

THE INFLUENCE OF ORGANIC ACIDS ON PHOSPHORUS AND ALUMINUM
RELEASE FROM SPODOSOLS OF THE SOUTHEASTERN COASTAL PLAIN

BY

THOMAS ROBERT FOX

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

1989

ACKNOWLEDGEMENTS

Throughout the course of my graduate program many people have provided assistance and support which, to a large degree, made this dissertation a reality.

First, I thank Nick Comerford, my advisory committee chairman, for his encouragement, intellectual stimulation and friendship. The other members of my advisory committee, Earl Stone, Brian McNeal, Pat Reid, Cliff Johnston, and T. L. Yuan also generously gave of their time, experience and expertise.

During his sabbatical in Florida, Bill McFee came to the rescue on many occasions. Mary McLeod helped in all phases of the lab work and in cutting through the mountains of red tape at the University. Steve Stearns-Smith allowed me to use his computer and printer, which made the final editing, if not a breeze, considerably easier. From the day I arrived in Gainesville, the friendship and help of Ken Van Rees made everything easier.

The financial support for this project was provided by Union Camp Corporation. I am extremely grateful to Bud Broerman, Research Manager for Union Camp, for his interest and support. Additional support was provided by the Intensive Management Practices Assessment Center (IMPAC) and the

Cooperative Research in Forest Fertilization (CRIFF) programs at the University of Florida.

I also thank my parents for their love, encouragement and understanding during this long process. Their pride in my work and accomplishments made getting this degree seem truly important.

David O'Keefe was a good friend with a kindred spirit who helped keep me sane during the insanity that is graduate school. Rover, my best buddy, put up with a lot of long hours on the rope after dark waiting for me to get home. He was always there with a wagging tail, which helped more than anyone can know. I hope we have more time for frisbee now.

Finally, I particularly want to thank Sue for her patience, love and understanding during the writing of this dissertation.

TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGEMENTS	ii
ABSTRACT	vi
CHAPTERS	
1 GENERAL INTRODUCTION	1
2 WATER SOLUBLE ORGANIC ACIDS IN SELECTED FOREST SOILS OF THE SOUTHEASTERN COASTAL PLAIN	13
Introduction	13
Materials and Methods	16
Results	26
Discussion	33
Summary	39
3 INFLUENCE OF OXALATE AND FORMATE ON THE KINETICS OF PHOSPHORUS AND ALUMINUM RELEASE FROM A FORESTED SPodosOL	41
Introduction	41
Materials and Methods	43
Results	49
Discussion	63
Conclusions	73
4 PHOSPHORUS AND ALUMINUM RELEASE FROM SPODIC HORIZON MATERIAL IN THE PRESENCE OF LOW-MOLECULAR-WEIGHT ORGANIC ACIDS	76
Introduction	76
Materials and Methods	78
Results	84
Discussion	89
Summary	95

5	INFLUENCE OF OXALATE LOADING RATE ON PHOSPHORUS AND ALUMINUM RELEASE FROM SPODIC HORIZON MATERIAL	97
	Introduction	97
	Materials and Methods	100
	Results	103
	Discussion	110
	Conclusions	113
6	THE EFFECT OF SEQUENTIAL OXALATE ADDITIONS ON PHOSPHORUS AND ALUMINUM RELEASE FROM TWO SPodosols OF THE SOUTHEASTERN COASTAL PLAIN	115
	Introduction	115
	Materials and Methods	116
	Results	120
	Discussion	131
	Conclusions	138
7	OVERALL CONCLUSIONS	140
APPENDIX		
	ACID PHOSPHATASE ACTIVITY AND PHOSPHATASE HYDROLYZABLE PHOSPHORUS IN TWO SPodosols	146
	REFERENCES	164
	BIOGRAPHICAL SKETCH	180

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

THE INFLUENCE OF ORGANIC ACIDS ON PHOSPHORUS AND ALUMINUM
RELEASE FROM SPodosOLS OF THE SOUTHEASTERN COASTAL PLAIN

By

Thomas Robert Fox

December 1989

Chairman: Nicholas B. Comerford
Major Department: Soil Science

The occurrence of oxalate and its effects on desorption of inorganic and organic P from surface and subsurface horizons of Spodosols of the southeastern United States is presented. Oxalate was the dominant organic acid identified in water extracts of soil, and its concentrations were higher in the spodic and argillic horizon soil. In the A horizon soils, oxalate had no effect on P desorption in comparison to water. In the subsoils, desorption of inorganic P increased by an order of magnitude in oxalate compared to water. Organic P release was also increased in the spodic horizon but not in the argillic horizon. In a comparison of 16 organic acids, a threshold value for $\log K_{Al}$ between 4.0 and 4.5 was required before substantial amounts

of inorganic P were released from the spodic horizon, after which release of inorganic P increased with increasing $\log K_{Al}$ values. For organic P, there was no correlation between the $\log K_{Al}$ value and the amount desorbed. The amount of inorganic P released from the spodic and argillic horizons increased as the oxalate loading rate (mmol oxalate/kg soil) increased. In the spodic horizons, organic P accounted for over 80 % of the P released in water. The percentage of organic P dropped as the oxalate loading rates increased. All extracts from the argillic horizons were essentially devoid of organic P. The effects on P release of a single oxalate addition were ephemeral, with most of the P that had been released being subsequently resorbed. The effects of repeated additions of oxalate were, however, cumulative. The amounts of both inorganic and organic P released depended only on the cumulative, total oxalate loading rate. The results presented have important implications for tree nutrition and forest productivity in the flatwoods. These data support the hypothesis that subsurface horizons could contribute a substantial portion of the P taken up by trees growing in the flatwoods.

CHAPTER 1 GENERAL INTRODUCTION

In acid soils, reactions with Fe and Al are generally thought to control P availability (Syers and Iskandar 1981). Phosphorus is specifically sorbed at the surface of Fe- and Al-hydroxides and at the broken edges of phyllosilicates (Sposito 1984). Phosphorus also reacts with Al complexed with humic and fulvic acids in soils (Levesque and Schnitzer 1967; Sinha 1971a, 1971b; Bloom 1980; Arp and Meyer 1985).

The mechanism of specific P adsorption by hydroxylated mineral surfaces has been intensively studied (Goldberg and Sposito 1985). The kinetics of adsorption (Hsu 1965; Kafkafi et al. 1967), hydroxyl release (Hingston et al. 1970, 1972, 1974; Ryden and Syers 1977) and infrared spectroscopic studies (Parfitt et al. 1977; Nanzyo 1984; Nanzyo and Watanabe 1984) all provide evidence indicating that ligand exchange is the mechanism of specific P adsorption. Ligand exchange involves the formation of an inner-sphere complex at a Lewis acid site of the Fe- and Al-hydroxide surface. Three types of inner-sphere P complexes at metal-hydroxide surfaces are possible: monodentate, bidentate and binuclear. The form of P on the soil surface may explain the difference between labile and non-labile P (Ryden and Syers 1975,

1977). The conversion from a monodentate to a bidentate form may transform sorbed P from a labile to a nonlabile (i.e. fixed) form (Kafkafi et al. 1967; Hingston et al. 1974). This explanation, however, remains controversial. Goldberg and Sposito (1985) contend that the evidence for the formation of bidentate P ligands is inconclusive. Diffusion of adsorbed P into Fe- and Al-oxides (an absorption reaction) has been suggested as an alternative mechanism of P fixation (Van Riemsdijk et al. 1984).

A large proportion of the total P in soils often exists in an organic form (Anderson 1980). The cycling of organic P has a large impact on P availability and long-term ecosystem productivity (Halstead and McKercher 1975). Soluble organic P is often a major component of the P released into soil extracts, sometimes exceeding the amount of inorganic P released. In a pioneering study by Pierre and Parker (1927), organic P contents of displaced soil solution from 20 soils averaged 0.015 mM, while inorganic P averaged 0.003 mM. Numerous studies since then have shown similar results (Martin 1964; Wild and Oke 1966; Martin 1970; Haynes and Swift 1985; Comerford and Skinner 1989). Turner and Lambert (1985) suggest that soluble organic P may be the most important form of P affecting long-term forest productivity.

The effects of organic anions on P reactions in soils have been studied for many years. In the 1940s, it was shown that P sorbed to Fe and Al could be replaced by organic

anions such as citrate and oxalate but not by others such as acetate and salicylate (Kurtz et al. 1946; Dean and Rubins 1947; Swenson et al. 1949; Low and Black 1950). Phosphorus fixation by Fe and Al was also reduced by the addition of certain organic anions (Struthers and Sieling 1950). These early workers observed that the relative effect of organic anions on P reactions was related to their ability to complex Fe and Al. Research since then has continued to show that organic anions that form stable complexes with Al and Fe increase P release from soil (Deb and Datta 1967; Nagarajah et al. 1968; Alexander and Robertson 1972; Appelt et al. 1975b; Lopez-Hernandez et al. 1979; Traina et al. 1986a, 1986b; Comerford and Skinner 1989).

Organic anions affect P availability through their reactions with Fe and Al both in solution and at oxide surfaces (Stumm 1986; Martell et al. 1988). Organic anions release P by direct ligand exchange reactions (Parfitt et al. 1977; Earl et al. 1979; Stumm and Morgan 1981) (fig. 1-1). Sorption of organic anions also changes the charge properties of the adsorbing metal-hydroxide surface which may affect P sorption (Hingston et al. 1967, 1970).

Organic anions that complex Al and Fe also stimulate the dissolution of Al and Fe oxide mineral surfaces (Huang and Violante 1986; Miller et al. 1986; Schwertmann et al. 1986; Tan 1986), which affects P availability.

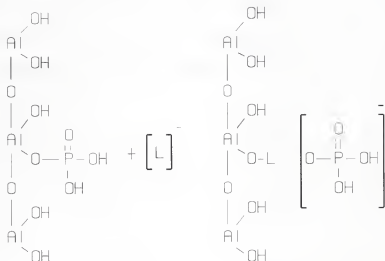
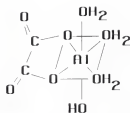


Figure 1-1. Ligand exchange reaction involving P at an Al-oxide surface. L represents a ligand capable of replacing H_2PO_4^- at the oxide surface.

Low-molecular-weight (LMW) organic acids affect metal solubility in soils through three mechanisms (Stumm 1986): 1) chelation of metals in solution reduces the activity of the free metal, and additional metal is released; 2) ligand exchange of organic acids at metal hydroxide surfaces tends to polarize the remaining metal-O bonds which become weaker, and thus metal dissolution increases; 3) the domain of congruent dissolution of the metal is increased by the presence of organic acids, allowing larger concentrations of metals to exist in solution without precipitation of another solid phase. Although the first mechanism is often assumed to be the most important, Stumm (1986) suggests that ligand exchange reactions and the formation of surface complexes is the primary mechanism of dissolution of metal hydroxides in soils. Organic anions in solution also retard the polymerization of Al and thus inhibit the precipitation of Al minerals (Ng Kee Kwong and Huang 1977; Goh and Huang 1984, 1985; Inoue and Huang 1984; Huang and Violante 1986).

The above reactions are predicated on the formation of stable organic anion-metal complexes (fig. 1-2). The formation of stable metal complexes depends on the presence and molecular arrangement of carboxylic and phenolic functional groups on the organic anion (Schnitzer and Skinner 1985; Martell et al. 1988). Stable complexes tend to involve five- and six-membered rings between the ligand and the metal (Cotton et al. 1987) (fig. 1-3).



Al-Oxalate Complex

Figure 1-2. Theoretical structure of an oxalate-aluminum complex

Stable Ring Structures



Five Member



Six Member

Figure 1-3. Five- and six-membered ring structures that impart stability to complexes

The magnitude of the stability constant ($\log K$) of a metal-ligand complex is a measure of the bond strength of the complex. The general form of a complexation reaction is given by



where M is a metal cation, L is a ligand and ML is the complex. The stability constant is calculated by

$$K = [ML]/[M][L] \quad [2]$$

where the brackets denote concentration (or more appropriately, activities) in solution.

The effects of organic acids on plant available P have been of recent interest (Graustein et al. 1977; Sollins et al. 1981; Gardner et al. 1983; Nye 1984; Jurinak et al. 1986; Marschner et al. 1986). Low-molecular-weight organic acids are important because they commonly occur in soils and are effective complexing agents. The most common organic acids in soil include acetic, citric, formic, fumaric, lactic, malic, oxalic, and succinic (Stevenson 1967; Bruckert 1970a, 1970b; Hue et al. 1986; Pohlman and McColl 1988). The concentration of organic acids in the bulk soil is quite variable, ranging from less than 10^{-6} to over 10^{-4} M (Stevenson 1967). Much higher concentrations, however, may occur at microsites near their point of origin, such as the rhizosphere (Smith 1969; Curl and Truelove 1986).

Organic acids in soils are produced by plants and microorganisms. Plant roots excrete large quantities of

organic acids into the soil (Vancura 1964; Smith 1969, Smith 1976; Gardner et al. 1982; Krafczyk et al. 1984). Leaching of organic acids from foliage and decomposing leaf litter is another important source (Muir et al. 1964a, 1964b; Bruckert 1970a; Kuiters and Sarink 1986; Pohlman and McColl 1988). Bacteria and fungi in the soil also produce large amounts of soluble organic acids (Bateman and Beer 1965; Maxwell and Bateman 1968; Graustein et al. 1977; Cromack et al. 1979; Malajczuk and Cromack 1982; Lynch 1983; Rozycki and Strzelczyk 1986).

The flatwoods of the southeastern Coastal Plain is an important timber growing region of the United States. The vegetation consists of slash pine (Pinus elliottii) with an understory dominated by gallberry (Ilex glabra), wax myrtle (Myrica cerifera) and saw palmetto (Serenoa repens) (Barbour and Billings 1988). Large amounts of organic acids are often produced in ecosystems with this type of vegetation (Stevenson 1967; Smith 1969; Bruckert 1970a, 1970b). Poorly drained Spodosols (Aquods) are the prevailing soil type in the flatwoods. The complexation and translocation of Al and Fe by organic acids is thought to be the primary mechanism involved in the podzolization process (Bloomfield 1953; Martin and Reeve 1960; Ponomereva 1969; Buol et al. 1980; Buurman 1984). These two facts suggest that organic acids are an important component of flatwoods forest ecosystems.

Plantations of slash pine are intensively managed for timber production throughout the flatwoods region. Productivity of these plantations is often limited by P availability and many pine stands in the flatwoods respond to P fertilization (Pritchett and Comerford 1983). Because the mineral fraction of these soils is dominated by quartz, organic matter plays an important role in nutrient availability. Organic P, therefore, may be an important component of the pool of available P.

In soils of the flatwoods, spodic and argillic horizons contain large amounts of Al which tends to fix the P in unavailable forms. These horizons tend to contain large quantities of total P; however, levels of water soluble P are extremely low. Organic acids that stimulate P release would increase the pool of available P in these soils. The presence of organic acids, therefore, may have important implications for forest productivity in the flatwoods. Slash pine roots tend to proliferate in subsurface horizons of Spodosols in the flatwoods (Van Rees and Comerford 1986). Phosphorus uptake from these subsurface horizons could make a significant contribution to tree nutrition (Comerford et al. 1984; Neary et al. In press).

Although high concentrations of organic acids would be expected, there is no information in the literature on the kinds and amounts of organic acids present in Spodosols of the flatwoods. There is also no direct information on the

effects of organic acids on P release from these soils. In addition, the levels of soluble organic P in extracts from these soils have not been investigated. Data on these topics are needed to improve our understanding of P fertility of Spodosols in the flatwoods.

The following research begins to address these needs. Each of the subsequent chapters investigates a particular facet of the effects of organic acids on release of both inorganic and organic P from Spodosols of the southeastern Coastal Plain. Each chapter is an independent manuscript eventually intended for journal publication.

Chapter 2 is a survey of the kinds and amounts of organic acids present in selected forest soils in the flatwoods of the southeastern Coastal Plain.

Chapter 3 examines the kinetics of P and Al release from surface, spodic and argillic horizons in the presence of the two most common organic acids identified in Chapter 2, oxalate and formate.

Chapter 4 is a comparison of the effects of a variety of organic acids on P and Al release from a spodic horizon. The objective was to test the hypothesis that P and Al release could be predicted based on the Al stability constant of the organic acids.

Chapter 5 is a study of the effects of differential oxalate loading on P and Al release. This study was needed because the effective oxalate concentration at the soil

surface may be much greater than the bulk solution concentrations described in Chapter 1.

Chapter 6 extends the information presented in Chapter 5 from a single oxalate addition to the effects of sequential oxalate additions on P and Al release. Organic acids are continuously produced in soils, and therefore it will be important to know the effects of repeated applications of oxalate on P release.

CHAPTER 2
WATER SOLUBLE ORGANIC ACIDS IN SELECTED FOREST
SOILS OF THE SOUTHEASTERN COASTAL PLAIN

Introduction

Organic acids alter chemical and biological processes in soils through complexation reactions with metals in solution and via ligand exchange reactions at soil surfaces (Stumm 1986; Martell et al. 1988). Historically, the emphasis in soils has been on chelation of Al and Fe in relation to soil genesis. The complexation and translocation of Al and Fe by organic acids is thought to be the primary mechanism involved in the podzolization process (Bloomfield 1953; Martin and Reeve 1960; Ponomereva 1969; Buurman 1984; Fisher and Yam 1984). Organic acids also retard the formation of crystalline soil minerals containing Al and Fe (Huang and Violante 1986; Schwertmann et al. 1986). Recently, interest in the effects of organic acids on solubility and speciation of metals such as Pb, Cd, Zn and Al has increased in response to environmental issues such as sludge application to soils and acid deposition (Sposito et al. 1980; Inskeep and Baham 1983; Inskeep and Comfort 1986; McColl and Pohlman 1986; Tam 1987; James and Riha 1989).

The availability of nutrients to plants, particularly P, is also increased in the presence of organic acids. (Elgawhary et al. 1970; Nagarajah et al. 1970; Fisher 1972; Earl et al. 1979; Gardner et al. 1983; Kraffczyk et al. 1984; Reid et al. 1985; Song and Huang 1988). Organic acids are an important component of the rhizosphere and contribute to increased nutrient availability in this region (Rovira and Davey 1974; Marschner et al. 1986; Jungk 1987).

Aluminum toxicity in acid soils may also be reduced by the presence of organic acids since Al in complexed form is not toxic to plants (Hue et al. 1986; Foy et al. 1987). Conversely, certain organic acids, particularly phenolic and fatty acids, are themselves toxic to plants and fungi when present at high concentrations (Jalal and Read 1983; Lynch 1983).

Organic acids are ubiquitous in forest and agricultural soils and a wide variety of organic acids have been identified (Stevenson 1967). The low-molecular-weight (LMW), aliphatic, organic acids commonly found in soils include oxalic, citric, formic, acetic, malic, succinic, malonic, maleic, lactic, aconitic and fumaric acids (Schwartz et al. 1954; Kaurichev et al. 1963; Whitehead 1964; Bruckert 1970a, 1970b; Cromack et al. 1979; Gardner et al. 1982; Jalal and Read 1983; Hue et al. 1986; Pohlman and McColl 1988). The specific organic acids present will determine the degree to which soil processes are affected.

Organic acids such as citric and oxalic, which form stable complexes with metals, will have a greater impact than those that do not form stable complexes, such as formic and lactic. The concentration of individual organic acids in soil solution ranges from less than micromolar to over millimolar (Stevenson 1967). Extremely high concentrations of organic acids can occur under special conditions, such as near roots of certain plant species or mats of fungal hyphae (Cromack et al. 1979; Gardner et al. 1983).

There are many sources of LMW organic acids in soil. The species of vegetation has a large effect on the amount and kinds of organic acids added to soils. Rainfall leaches organic acids from the leaves and bark of living plants and from decomposing plant litter in the forest floor (Bloomfield 1953; Muir et al. 1964a; Kuiters and Sarink 1986; Pohlman and McColl 1988). Plant root exudates are another important source of organic acids in soil (Vancura 1964; Vancura and Hovadik 1965; Smith 1969; Gardner et al. 1982; Kraffczyk et al. 1984). Fungi, including mycorrhizae, bacteria and actinomycetes also release organic acids into the soil (Fisher 1972; Cromack et al. 1979; Malajczuk and Cromack 1982; Lynch 1983; McKeague et al 1986; Rozycki and Strzelczyk 1986). Low-molecular-weight organic acids also arise as decomposition products of larger organic compounds (Stevenson 1982). Organic acids of the fatty acid sequence (formic, acetic, butyric) often accumulate in anaerobic

environments as a result of incomplete decomposition of organic residues added to soil (Stevenson 1967; Reddy et al. 1986)

The flatwoods region of Florida and southern Georgia is characterized by poorly drained Spodosols (Aquods) that support stands of slash pine with a shrub understory dominated by gallberry, wax myrtle and saw palmetto. Large amounts of organic acids are usually produced in ecosystems with this type of vegetation (Stevenson 1967; Smith 1969; Bruckert 1970a, 1970b). The well-developed Spodosols in this region also suggest that organic acids are, or have been, an important component of flatwoods forest ecosystems (Ponomereva 1969; Buurman 1984). In spite of this, there is no information in the literature on the kinds and amounts of LMW organic acids in soils of the flatwoods. In light of the significant effects that organic acids can have on soil and ecosystem processes, knowledge of the suite of organic acids present in these soils would be valuable. The purpose of this study was to characterize the LMW, aliphatic, organic acids present in selected soils of the flatwoods.

Materials and Methods

Water-soluble, LMW, aliphatic organic acids in forest soils of the Florida flatwoods were measured in three separate studies.

Experiment 1

In the first study, five sites in Alachua County, Florida were sampled during November 1988 (Table 2-1). The sites were selected to include a variety of vegetation and soil types. The Candler soil (hyperthermic, uncoated, Typic Quartzipsamment) is a deep, excessively drained sand of very low fertility. The vegetation is typical of a xeric sandhill site. The Lochloosa soil (loamy, siliceous, hyperthermic, Aquic, Arenic Paleudult) has a sandy cap over an argillic horizon and supports a slash pine plantation with a more mesic understory vegetation. Three sites with Pomona soil (sandy, siliceous, hyperthermic, Ultic Haplaquod) supporting pine plantations of different ages and species were sampled. The Pomona series is widespread in the flatwoods and is characterized by a spodic horizon over an argillic horizon and a fluctuating water table. Understory vegetation was more typical of the flatwoods (Table 2-1).

Samples from the forest floor and the surface 15 cm of mineral soil were collected from a single location at each site. Samples were placed in polyethylene-lined paper bags and kept on ice during transport to the laboratory. After storage overnight at 3 °C, forest floor samples were ground in a coffee grinder and soil samples were passed through a 2-mm sieve. Duplicate, 10-g samples of ground litter or sieved soil were placed in disposable plastic filter flasks fitted with 0.45 micron nylon membrane filters.

Table 2-1. Soil and vegetation characteristics of forested sites in Alachua County, Florida, sampled in October 1988.

Soil Series	Drainage	Overstory			Origin	Understory Species
		Species	Age	Basal Area		
			(yr)	(M ² /ha)		
Candler	Excessive	Longleaf pine Turkey Oak	40	5	Natural	WG
Lechloosa	Some-what Poor	Slash pine	32	22	Plantation	SP, WM
Panosa	Poor	Slash pine	23	25	Plantation	GB, SP, WM, L
Panosa	Poor	Slash pine	6	-	Plantation	GB, WM, L
Panosa	Poor	Slash pine	6	-	Plantation	GB, WM, L

1. WG=wire grass; SP=saw palmetto; WN=wax myrtle; GB=gallberry; L=lyonia

Twenty mL of distilled water adjusted to pH 4.3 with HCl were added to each flask and allowed to leach under gravity for 12 hours. The flasks were then attached to a vacuum pump and residual leaching solution was extracted under a vacuum of 635 mm Hg for 15 minutes. All leachate was collected and analyzed for organic acids the same day.

Experiment 2

Following the initial survey of LMW organic acids in a cross section of flatwoods soils, a more intensive study of organic acid distribution within a single soil type was conducted. Eight sites with Pomona series soil were selected (Table 2-2). Slash pine plantations ranging from 23 to 30 years of age occurred on four sites, while natural stands of slash and longleaf pine 47 to 63 years old occurred on the other four sites. Understory vegetation on all sites was similar and was dominated by saw palmetto, gallberry and wax myrtle.

Soil samples from the A and Bh horizons were collected in each stand during January 1989. Composite samples, consisting of material from three bucket-auger holes, were placed in polyethylene-lined paper bags and kept on ice during transport to the laboratory. The gravimetric moisture content of each sample was determined immediately (Gardner 1986). After storage overnight at 3 °C, soil solution was obtained by centrifugation (Adams et al. 1980;

Table 2-2. Characteristics of pine stands on Pomona series soil in Alachua County, Florida, sampled in January 1989.

Location	Overstory			Understory Species
	Species	Age	BA	
		(yr)	(m ² /ha)	
Plantation 1	slash	25	23	SP, GB
Plantation 2	slash	23	25	SP, GB
Plantation 3	slash	26	21	SP, WM, GB
Plantation 4	slash	30	28	GB, SP
Natural Stand 1	slash longleaf	55	11	GB, SP, WM, CB
Natural Stand 2	slash longleaf	63	22	SP, WG
Natural Stand 3	slash longleaf	47	23	SP, WM, WG
Natural Stand 4	slash	49	18	GB, SP

1. SP=saw palmetto; WG=wire grass; WM=wax myrtle; GB=gallberry;
CB=chalky bluestem

Elkhatib et al. 1987). Two hundred grams of field-moist soil were placed in a plexiglass centrifuge bottle fitted with a perforated bottom and a second receiving receptacle and centrifuged at 1000 x g for 15 minutes. The extracted soil solution was filtered through 0.45 micron Luer-lock cellulose acetate filter disks. The filtrate was analyzed the same day for organic acids. Organic acid concentrations were adjusted to 20 % (w/w) soil moisture content to eliminate dilution effects arising from differences in the initial moisture content of the soil.

Experiment 3

Changes in the organic acid content of the rhizosphere of slash pine were investigated in a third experiment. Soil from surface and subsurface horizons of two Spodosols were used for this study. The first soil was a Pomona series located in Alachua County, Florida. The second soil was a Leon series (sandy, siliceous, thermic Aeric Haplaquod) from Charlton County, Georgia. The major difference between the two soils is the presence of an argillic horizon below the spodic horizon in the Pomona series. Soil samples were collected from individual pedogenic horizons (Leon A and Bh; Pomona A, Bh, Bt) from a single soil pit dug for each series. The soils were air-dried, passed through a 2-mm sieve, and stored in plastic barrels prior to the experiment.

Triplicate, 3-kg samples of air-dried soil from a specific horizon were placed in individual 15-cm diameter by 15-cm deep plastic pots. The soil in each pot was wetted to saturation with tap water and allowed to drain for two weeks.

Half-sib seed from genetically improved slash pine (UF 84-57) were obtained from the Cooperative Forest Genetics Research Program at the University of Florida. The seeds were soaked in distilled water for 24 hours and then placed on moist perlite in an enclosed plastic chamber and allowed to germinate. Seedlings were transplanted to the pots after primary needles had emerged but before the seed coats were shed. Six seedlings were planted per pot and were thinned to 4 seedlings per pot after 2 weeks. Seedlings were grown for 6 months (January through June 1988) in a greenhouse in Gainesville, Florida under natural light. Pots were watered as needed with tap water and excess water was allowed to drain by gravity. At monthly intervals, 100 mL of a "minus P" nutrient solution ($0.004 \text{ M Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}$; $0.002 \text{ M (NH}_4)_2\text{SO}_4$; 0.006 M KNO_3 ; and $0.001 \text{ M MgSO}_4 \cdot 7\text{H}_2\text{O}$) were added to each pot. Seedlings were sprayed with malathion in February to control aphids and in May to control spider mites.

After 6 months, the seedlings were removed from the pots. The entire root system from all four seedlings in each pot was treated as a single unit. Rhizosphere soil was separated from bulk soil by gently shaking the root system.

Soil adhering to the root system was defined as rhizosphere soil while the non-adhering soil was defined as bulk soil (Rovira and McDougall 1967). Rhizosphere soil was subsequently separated from the roots by a more vigorous shaking. Soil still adhering to the roots was removed by hand with a dissecting needle.

Rhizosphere and non-rhizosphere soil from each pot was placed in separate polyethylene plastic bags and immediately placed on ice. The rhizosphere and bulk soil samples were stored overnight at 3 °C. Analyses were initiated the following day.

Organic acids were leached from the soil with distilled water adjusted to pH 4.3, using the same procedure as in experiment 1. Analyses were performed on field-moist soil samples. Gravimetric moisture content of each soil was determined (Gardner 1986) and used to correct for differences in the initial soil moisture content of each soil.

Organic Acid Analysis

Low-molecular-weight aliphatic organic acids in the filtered solutions were analyzed by isocratic high performance liquid chromatography (HPLC). The HPLC system consisted of a Gilson single-piston pump and pressure module (Gilson Medical Electronics, Inc., Middleton, Wisconsin), a Rheodyne model 7125 injection valve fitted with a 20 μ L injection loop (Rheodyne, Inc., Cotati, California), a

Hamilton PRP-X300 150 x 4.1 mm organic acid column (Hamilton Co., Reno, Nevada), a Gilson Holochrom variable wavelength uv detector and a Gilson computerized integrator. Organic acids were separated at ambient temperature with H_2SO_4 as the eluent at a flow rate of 2 mL/min and detected at 210 nm with the range selector set at 0.05 AU full scale. The partially sulfonated poly(styrene-divinylbenzene) stationary phase of the PRP-X300 column utilizes a combination of reverse-phase, hydrogen bonding and ion-exclusion mechanisms to achieve separation (Lee and Lord 1986). Since eluent pH has a strong affect on the retention characteristics of organic acids on this column, duplicate analyses at two pH values, obtained by varying the H_2SO_4 concentration in the eluent, were used to enhance separation of coeluting compounds. Organic acids were identified by comparing retention times of unknowns with those of reagent-grade organic acid standards at the two eluent concentrations (Table 2-3). Organic acids were quantified by comparing peak areas with an external standard curve.

Statistical Analysis

All statistical analyses were conducted using the Statistical Analysis System (SAS). Computing was done using the facilities of the Northeast Regional Data Center of the State University System of Florida, located on the campus of the University of Florida in Gainesville.

Table 2-3. Retention times of selected aliphatic organic acids (Hamilton PRP-X300 4.2 x 150 mm column, flow rate 2 mL/min) with sulfuric acid as the eluent at two concentrations.

Organic Acid	Eluent H ₂ SO ₄ Concentration	
	0.001N	0.02N
	(minute)	
Oxalic	0.28	0.38
Tartaric	0.35	0.43
Lactic	0.44	0.86
Malic	0.49	0.61
Maleic	0.49	1.48
Malonic	0.50	0.76
Formic	0.52	0.57
Citric	0.58	0.83
Acetic	1.20	1.22
Aconitic	1.35	2.63
Fumaric	1.81	3.09
Succinic	1.71	1.98

Means and standard deviations were used to summarize the data from the initial survey of organic acids. In the second study, analysis of variance (ANOVA) was used to test for differences in organic acid concentrations 1) between natural stands and plantations and 2) between A and Bh horizons. Data were analyzed as a 2x2 factorial with 4 replications in a completely randomized design. Analysis of variance (ANOVA) was used to test for differences among soils and between rhizosphere and non-rhizosphere soil in the third study. The data were analyzed as a split plot experiment with soil horizon as the main plot and rhizosphere vs non-rhizosphere as subplots, in a randomized complete block design with 3 replications (Gomez and Gomez 1984).

Results

Experiment 1

The mosaic of LMW organic acids present in the forest floor leachates was similar among the five sites (Table 2-4). Oxalic and formic acids were present in all samples at relatively high concentrations. Formic acid concentrations, at up to millimolar levels, were greater than those of oxalic acid. Citric and acetic acids were also found in all samples but their concentrations were much lower. Malic, lactic and aconitic acids were detected in

Table 2-4. Low molecular weight aliphatic organic acids in forest floor leachates from sites in Alachua County, Florida, sampled in November 1988.

Soil	Oxalic	Formic	Citric	Acetic	Malic	Lactic	Acetonic
			(micro moles/liter)				
Candler	28(9) ¹	tr ²	9(2)	tr	.3	-	-
Lechloose	110(8)	1428(24)	tr	5(2)	-	tr	tr
Panama 1	26(0)	tr	3(3)	tr	-	-	-
Panama 2	58(1)	631(85)	tr	tr	-	-	-
Panama 3	119(6)	1475(67)	tr	6(1)	tr	-	tr

1. Mean value of duplicate samples are followed by standard deviations in parenthesis.

2. Trace amounts: peak identified at appropriate retention times but peak area not integrated.

3. No peak identified at appropriate retention time.

only two of the samples, and their concentrations were near the detection limit.

The mixture of organic acids in the A horizon mineral soil leachates was slightly less diverse than in the forest floor (Table 2-5). Oxalic and formic acids were again present in all soils and occurred in the highest concentrations. Citric, acetic and aconitic acids were found at low concentrations in some of the soils whereas lactic and malic acids were not detected.

Experiment 2

The organic acids identified in centrifuged soil solution from surface and spodic horizons included oxalic, formic, citric, acetic, malic, and succinic (Table 2-6). Oxalic and formic acids predominated in all soils. Oxalic acid concentrations approached millimolar levels and were generally higher than those for formic acid. Traces of citric acid were present in all soils. The remaining organic acids did not consistently appear in all soils and, when present, occurred in only trace amounts. Oxalic acid concentrations in the Bh horizons were greater than in the surface horizons (Table 2-7). No other significant differences were detected.

Experiment 3

As in the previous experiments, the only organic acids detected in significant quantities were oxalic and formic (Table 2-8). There were no differences in oxalic acid

Table 2-5. Low molecular weight aliphatic organic acids in surface soil leachates from sites in Alachua County, Florida, sampled in November 1988.

Soil	Oxalic	Formic	Citric	Acetic	Malic	Lactic	Aconitic
			(micro moles/liter)				
Candler	85(20) ¹	717(67)	tr ²	tr	.3	-	-
Lochloosa	304(20)	1142(113)	tr	tr	-	-	tr
Panama 1	525(346)	tr	-	tr	-	-	tr
Panama 2	209(37)	tr	tr	-	-	-	-
Panama 3	231(48)	473(473)	tr	-	-	-	-

1. Mean values of duplicate samples are followed by standard deviations in parenthesis.

2. Trace amounts: peak identified at appropriate retention times but peak area not integrated.

3. No peak identified at appropriate retention time.

Table 2-6. Low molecular weight aliphatic organic acids identified in soil solution from Pomona series soils supporting pine stands in Alachua County, Florida, sampled in January 1989.

Stand	Horizon	Oxalic	Formic	Citric	Acetic	Malic	Succinic
(micro moles/liter)							
Plantation 1	A	193	19	tr ¹	tr	.2	-
	Bh	362	32	tr	-	-	tr
Plantation 2	A	62	63	tr	tr	-	-
	Bh	603	148	tr	tr	tr	tr
Plantation 3	A	125	89	tr	-	-	-
	Bh	313	45	tr	-	-	tr
Plantation 4	A	293	64	tr	tr	tr	tr
	Bh	1043	77	tr	-	-	-
Natural 1	A	358	tr	-	-	-	-
	Bh	142	19	tr	tr	tr	tr
Natural 2	A	98	174	-	-	-	-
	Bh	460	6	tr	tr	tr	-
Natural 3	A	154	151	tr	tr	tr	-
	Bh	733	137	tr	tr	-	tr
Natural 4	A	198	9	tr	-	-	-
	Bh	465	5	tr	tr	tr	tr

1. Trace amounts: peak identified at appropriate retention times but peak area not integrated.

2. No peak identified at appropriate retention time.

Table 2-7. Mean value contrasts of low molecular weight aliphatic organic acids identified in soil solution from Pomona series soils supporting pine stands in Alachua County, Florida, sampled in January 1989.

Sample	n	Oxalic	Formic
(micro mole/liter)			
Comparison between stand types:			
Plantation	8	326	63
Natural Stand	8	284	67
		ns	ns
Comparison between soil horizons:			
A Horizon	8	185	71
Bh Horizon	8	525	59
		*	ns

ns Differences between mean values not significantly different ($\alpha=0.05$).

* Difference between mean value significantly different ($\alpha=0.05$).

Table 2-8. Low- molecular-weight, aliphatic, organic acids identified in extracts of rhizosphere and bulk soil from pots growing slash pine seedlings.

Soil Horizon	Location	Oxalic	Formic	Citric	Acetic	Aconitic	Total
(micro mole/liter)							
Leon A	Rhizosphere	202	677	tr ¹	tr	tr	880
	Bulk	221	.2	tr	-	-	221
Leon Bh	Rhizosphere	2810	638	tr	tr	tr	3498
	Bulk	2690	-	-	-	-	2690
Panama A	Rhizosphere	222	349	tr	tr	tr	571
	Bulk	552	-	-	-	-	552
Panama Bh	Rhizosphere	2052	2724	tr	tr	tr	4776
	Bulk	2191	-	-	-	-	2191
Panama Bt	Rhizosphere	2200	918	tr	tr	tr	3117
	Bulk	2937	-	-	-	-	2937
L80 (0.05) Horizons		1639	1404				1813
L80 (0.05) Locations		1434	-				2190

1. Trace amount: peak identified at appropriate retention time but peak area not integrated.

2. No peak identified at appropriate retention time.

concentrations between the rhizosphere and non-rhizosphere soil. However, qualitative differences in the suite of organic acids existed between the rhizosphere and bulk soil (Table 2-8). With the exception of the Leon A horizon, where trace amounts of citric acid were detected, oxalic acid was the only aliphatic organic acid detected in the non-rhizosphere soil. In contrast, oxalic, formic, citric, acetic and aconitic acids were detected in all samples of rhizosphere soil. The most obvious difference between the rhizosphere and bulk soils was the relatively large concentrations of formic acid in the rhizosphere. Although total organic acid concentrations were consistently higher than in the rhizosphere, only in the Pomona Bh horizon was the total concentration of aliphatic organic acids in the rhizosphere significantly greater than in the bulk soil (Table 2-8).

The oxalic acid and total aliphatic organic acid concentrations in both the rhizosphere and bulk soils were higher in the Bh and Bt horizons than for the A horizon soil (Table 2-8). Formic acid was also higher for the Bh horizon in the Pomona series but not in the Leon series.

Discussion

Low-molecular-weight organic acids were abundant in the forest floor, soil and rhizosphere of the forest ecosystems from the flatwoods of the southeastern Coastal Plain

included in this study. Oxalic and formic acids were the dominant organic acids identified. This is not surprising given the type of vegetation in this region. Oxalic acid has frequently been found in abundance in litter and mineral soil extracts from forest ecosystems. This includes ecosystems dominated by spruce in Russia (Kaurichev et al. 1963); oak, beech, pine and fir in France (Bruckert 1970a, 1970b); and incense cedar, ponderosa pine, and Douglas-fir in the western United States (Pohlman and McColl 1988). Oxalic acid concentrations high enough to exceed the solubility of Ca-oxalate ($pK_{sp}=8.64$) have been found in soil under mats of fungi (Hysterangium crassum) in Douglas-fir stands in Oregon (Cromack et al. 1979) and in association with ectomycorrhizal roots of Pinus radiata and Eucalyptus marginata in Australia (Malajczuk and Cromack 1982).

The occurrence of large concentrations of formic acid in soils has been reported much less frequently in the literature. The cause for this may relate to the methodology employed to identify organic acids. Until recently, thin layer chromatography was the principle method of organic acid analysis, and formic acid often could not be determined since a solution of diethyl ether/formic acid was used to develop the chromatograph (Smith 1969). Using alternative methods, formic acid was found to be a dominant organic acid in extracts from soils in Ohio supporting virgin hardwood forests (Schwartz et al. 1954). Along with oxalic acid,

Kaurichev et al. (1963) also found substantial amounts of formic acid in litter from spruce forests. Hue et al. (1986) found higher levels of formic than oxalic acid in forested soils in Alabama.

The mixtures of organic acids in forest soils are generally quite diverse. Citric, acetic, malic, lactic, aconitic and succinic acids were also often found in the soil and litter samples in this study. The concentrations of these compounds were, however, always much lower than those of oxalic and formic acids. The suite of aliphatic acids from the present and other published studies in the literature are summarized in Table 2-9.

The presence of large quantities of organic acids in the ecosystems in this study was not unexpected. Oxalic acid is produced in natural systems by a large number of plants and microorganisms, including fungi and bacteria (Hodgkinson 1977). In certain genera of fungi, such as Aspergillus, calcium oxalate may account for up to 45 % of the dry weight of the fungal mycelium (Hodgkinson 1977). Oxalic acid is also a primary component of the root exudates of trees, including pines (Smith 1969; Smith 1976). As the simplest fatty acid, formic acid is also an extremely common organic acid in nature. In addition to its synthesis in plants and microorganisms, formic acid is a common product of organic matter decomposition in anaerobic environments (Stevenson 1967). The relatively large concentrations of formic acid

Table 2-9. Summary of aliphatic organic acids identified under differing soil and vegetation conditions.

Organic Acid	This Study	Pohlman & McColl, 1988	Bruckert, 1970a	Schwartz et al., 1954	Hue et al., 1986
Acetic	+		+	+	
Aconitic	+	+			
Citric	+		+		+
Formic	+			+	+
Fumaric		+			
Lactic	+		+		+
Maleic		+			
Malic	+		+		+
Malonic			+		+
Oxalic	+	+	+		+
Succinic	+		+		+

may also be indirectly related to the large oxalic acid production in these ecosystems. Stoichiometric quantities of formic acid and CO_2 are produced during the decarboxylation of oxalic acid by oxalate decarboxylase enzyme (Hodgkinson 1977)



This enzyme is produced by several species of wood-decaying fungi in the soil and is most active in acid environments (Hodgkinson 1977). The optimum pH range for these enzymes, from 3.0 to 5.2, brackets the pH values for the soils of this study. Bacteria in the soil may also utilize oxalate as a carbon source and, through a series of reactions involving coenzyme A (CoA), produce formic acid (Hodgkinson 1977; Allison and Cook 1981).

Release of root exudates and production by fungi and bacteria, which are more active in the rhizosphere (Rovira and Davey 1974), probably accounts for the more diverse and higher total organic acid concentrations found in the rhizosphere soil (Table 2-8). The high concentrations of formic acid in the rhizosphere may be caused by anaerobic decomposition of organic matter. Higher rates of respiration occur in the rhizosphere and may lead to the development of anaerobic microsites (Rovira and McDougall 1967; Curl and Truelove 1986). Formic acid may accumulate under these conditions (Stevenson 1967).

The greater oxalic acid concentrations found in Bh and Bt horizons in both the field and in the greenhouse pot studies suggests that either greater production occurs in the Bh and Bt horizons or that degradation is inhibited in these horizons. Greater amounts of root exudates are usually produced in response to nutrient and other environmental stresses (Curl and Truelove 1986). Bowen (1969) found increased production of root exudates for radiata pine grown in a phosphorus-deficient nutrient solution. Likewise, greater amounts of oxalic acid may be exuded by slash pine roots in the Bh and Bt horizons where levels of water soluble P are low. Increased production of organic acids in the roots of Al tolerant strains of barley occurred in response to elevated Al levels in nutrient solutions (Foy et al. 1987). These authors suggested that organic acid production by plant roots may be a mechanism of Al tolerance. Increased oxalate production by the roots of slash pine may occur in the Bh and Bt horizons in response to high amounts of Al, as a mechanism protecting them from Al toxicity. Differential rates of oxalic acid degradation between the surface and spodic horizons may also contribute to the higher concentrations of oxalate in the Bh horizons. Oxalate forms extremely strong complexes with Al. In complexed form, the resistance of oxalate to microbial attack may be increased in the same way that other organic matter complexes are stabilized in soil (Martin and Haider

1986). This process would be more pronounced in the Bh and Bt horizons, where the amounts of Al are much higher than in the A horizon.

Because of its ability to strongly complex metals such as Al, the presence of large quantities of oxalate in soils of the flatwoods has important implications for soil chemistry and plant nutrition. Oxalate may increase P availability to plants in these soils by complexing Al, particularly in the Bh and Bt horizons. The presence of oxalate in soil solution also alters metal speciation, which may have significant effects on current environmental chemistry problems such as acid deposition and heavy metal contamination of soils (Sposito et al. 1980; Johnson et al. 1982; Inskeep and Baham 1983; Martell et al. 1988; James and Riha 1989). Further work on the role of oxalate in these ecosystems seems appropriate.

Summary

LMW organic acids alter biochemical processes in the soil that control metal speciation, solubility and nutrient availability. This study was conducted to identify and quantify the LMW, aliphatic, organic acids present in forested Spodosols of the flatwoods. Water soluble organic acids in forest floor litter, surface and spodic horizon soil, and the rhizosphere of slash pine seedlings were determined by HPLC. Oxalic acid was found in all samples and

was generally present in the highest concentration, ranging from 25 to 3000 μM . Oxalic acid concentrations were greater in the Bh and Bt horizons than in the A horizon soil. Formic acid was also identified in large concentrations (5 to 1500 μM) in most of these soils. Trace amounts of citric, acetic, malic, lactic, aconitic, and succinic acids were also detected in some samples. In the rhizosphere of slash pine seedlings grown in pots, the suite of organic acids identified was more diverse than in the bulk soil. The presence of large concentrations of oxalate in the soils of the flatwoods may have important implications for P availability in these soils which tend to be dominated by Al.

CHAPTER 3
INFLUENCE OF OXALATE AND FORMATE ON THE KINETICS OF
PHOSPHORUS AND ALUMINUM RELEASE FROM A FORESTED SPodosol

Introduction

The influence of low-molecular-weight (LMW) organic acids on P availability has drawn considerable attention in recent years (Graustein et al. 1977; Sollins et al. 1981; Gardner et al. 1983; Marschner et al. 1986; Jurinak et al. 1986). Because LMW organic acids function as organic ligands, they can increase P availability by replacing P sorbed at metal-hydroxide surfaces through ligand-exchange reactions (Berkheiser et al. 1980; Stumm 1986). They also complex metals in solution and thus prevent precipitation of metal phosphates (Ng Kee Kwong and Huang 1977). Complexation reactions at surfaces and in solution also increase dissolution of metal-oxide surfaces (Stumm and Morgan 1981; Martell et al. 1988). The above reactions are predicated on the formation of metal-organic-acid complexes more stable than a metal-P complex. The stability of metal-organic-acid complexes depends on the presence and molecular arrangement of carboxylic and phenolic functional groups on the organic acid (Martell et al. 1988). Stable complexes tend to possess a five- or six-membered ring structure (Cotton et al. 1987).

In Spodosols of the southeastern United States, reactions with Al tend to control P availability (Ballard 1973; Ballard and Fiskell 1974). The relative effect of LMW organic acids on P reactions in these soils, therefore, depends on their ability to complex Al. Organic acids that form stable complexes with Al should have a greater effect on P desorption.

The presence of chelating organic ligands in soils may affect both the equilibrium conditions and the kinetics of P release. Kuo and Lotse (1974) reported that the rate of P desorption from gibbsite was faster in the presence of EDTA than in the presence of F. Likewise, Traina et al. (1987) observed a more rapid initial release of P from an acid, montmorillonitic soil in the presence of citrate compared with formate. In both cases, the rate of P release was faster with the ligand that formed more stable complexes with Al.

In Chapter 2, oxalate and formate were found to be the dominant LMW organic acids in a group of representative Spodosols studied. Since oxalate forms more stable complexes with Al than does formate (Martell and Smith 1977), P desorption should be affected to a larger degree by oxalate than by formate. Oxalate may, therefore, have significant effects on P nutrition of trees growing in the flatwoods, where P availability often limits forest productivity. Since the soil solution P content must be replenished to meet the

P uptake rate of plants, knowledge of the kinetic pattern of P desorption will be needed to estimate the ability of these soils to supply P. This chapter presents an initial investigation into the effects of oxalate and formate on P availability. The objectives of this study were to examine the kinetics of P and Al release from A, Bh and Bt horizon soil material from a representative, poorly-drained, flatwoods Spodosol.

Materials and Methods

Soil Material

Samples of soil material from A, Bh and Bt horizons from a Pomona series (sandy, siliceous, hyperthermic Ultic Haplaquod) soil were collected from a single soil pit located in Alachua County, Florida. The soil material was air-dried, passed through a 2-mm sieve, and stored in plastic barrels prior to use. Selected chemical and physical properties, determined by standard methods (Page et al. 1982; Klute 1986), are presented in Table 3-1.

Desorption Experiments

The kinetics of P desorption in oxalate, formate and water were investigated using a batch procedure. The properties of oxalic and formic acid are listed in Table 3-2. Oxalate solutions of 0.1 mM and 1.0 mM were prepared from reagent-grade oxalic acid, monohydrate. Millimolar formate solutions were prepared from reagent-grade Ca-formate.

Table 3-1. Selected physical and chemical characteristics of the Pomona series soil.

Property	Horizon		
	A	Bh	Bt
Sand (%)	93	89	78
Clay (%)	1	4	18
Organic C (%)	1.77	2.19	0.31
pH (H ₂ O)	4.03	4.23	4.74
<u>Phosphorus</u>			
Total P (mg/kg)	21.8	65.5	138.6
Total Organic P (mg/kg)	9.6	49.2	27.1
Mehlich 1 (mg/kg)	5.1	7.6	9.7
0.01 M CaCl ₂ (mg/kg)	7.0	2.0	1.0
Distilled Water (mg/kg)	9.1	0.4	0.1
<u>Potassium</u>			
Mehlich 1 (mg/kg)	13	3	2
<u>Calcium</u>			
Mehlich 1 (mg/kg)	111	13	14
<u>Magnesium</u>			
Mehlich 1 (mg/kg)	30	4	8
<u>Aluminum</u>			
Acid Oxalate (mg/kg)	226	1357	1075
Pyrophosphate (mg/kg)	215	1379	769
Mehlich 1 (mg/kg)	37	432	230
1 N KCl (mg/kg)	15	158	118
0.01 M CaCl ₂ (mg/kg)	10	97	42
Distilled Water (mg/kg)	2	13	3
<u>Iron</u>			
Acid Oxalate (mg/kg)	12	6	9
Pyrophosphate (mg/kg)	35	19	89
Mehlich 1 (mg/kg)	3	2	22
0.01 M CaCl ₂ (mg/kg)	2	2	5
Distilled Water (mg/kg)	0.7	0.6	0.8

Table 3-2. Physical and chemical properties of oxalic and formic acids.

Acid	Oxalic Acid	Formic
Formula	$\text{HO}_2\text{CCO}_2\text{H}$	HCO_2H
Formula Wt.	90.04	46.03
Dissociation Const. (pK)	1.23; 4.19	3.75
Ligand	H_2L	HL
Stability Const. ($\log K_{AL}$)	6.10	1.36

Deionized water was also used. The organic acid solutions and the deionized water were adjusted to pH 4.3 with HCl or NaOH. The solutions had the following ionic strengths: 1.0 mM oxalate = 0.0031 M; 0.1 mM oxalate = 0.0008 M; 1.0 mM formate = 0.0029 M; pH 4.3 acidified water = 0.0001 M.

Duplicate, 10-g samples of air dried soil material were placed in 250-mL polyethylene bottles and 100 mL of extracting solution were added to the bottle along with two drops of toluene. The bottles were capped and shaken on a reciprocating shaker at 100 cycles per minute. Samples were removed after 0.5, 1, 3, 6, 12, 24, 48 and 72 hours and filtered through 0.45 micron nylon membrane filters. The filtrates were frozen at 0°C until analyzed.

Chemical Analysis

The conductivity of the filtered extracts was measured with a platinum conductivity electrode on a YSI conductivity meter. Solution pH was measured with a combination glass electrode on an Orion pH meter. Aluminum was determined using flame emission spectrophotometry with a $N_2O-C_2H_2$ flame. In selected samples, Fe, K, Ca, Na, Mg, Zn, Mn, and Cu were also determined by inductively-coupled plasma emission spectrophotometry.

Inorganic P in the filtrate was determined by a molybdenum-blue colorimetric procedure using ascorbic acid as a reductant (Murphy and Riley, 1962). This is an operational definition of inorganic P, since molybdenum

hydrolyzes some organic P compounds (Stainton 1980, Tarapchak et al. 1982). Total P was also measured in each sample as follows: A 20-mL aliquot of the filtrate was evaporated to dryness at 100 ° C in a 50-mL pyrex beaker. The beaker was than placed in a muffle furnace overnight at 500 °C. The beaker was allowed to cool and then placed on a hot plate. A 10-mL aliquot of 40 % HCl was added to each sample and evaporated to dryness followed by a 5-mL aliquot of concentrated HCL which was also evaporated to dryness. The beakers were allowed to cool and the sample was redissolved in 20 mL of 0.1 N HCl. Phosphorus in solution was again measured by the Murphy-Riley procedure. Soluble organic P was operationally defined as the difference between Murphy-Riley P in the dry-ashed and acid-digested sample (total P) and Murphy-Riley P in the initial leachate (inorganic P).

Organic Acid Analysis

LMW, aliphatic organic acids in the filtered solutions were analyzed by isocratic high performance liquid chromatography (HPLC) (Lee and Lord 1986). The HPLC system consisted of a Gilson single-piston high-pressure pump and pressure module (Gilson Medical Electronics, Inc., Middleton, Wisconsin), a Rheodyne model 7125 injection valve fitted with a 20 µL injection loop (Rheodyne, Inc., Cotati, California), a Hamilton PRP-X300 150 x 4.1 mm organic acid column (Hamilton Co., Reno, Nevada), a Gilson Holochrom

variable-wavelength uv detector and a Gilson computerized integrator. Organic acids were separated at ambient temperature with H_2SO_4 as the eluent at a flow rate of 2 mL/min and detected at 210 nm. Organic acids were quantified by comparing peak areas with an external standard curve.

Statistical Analysis

Statistical differences in the pattern of P desorption kinetics were tested using a regression procedure. The observed relationship between P desorption and time suggested a cubic equation of the form:

$$P \text{ desorbed} = \beta_0 + \beta_1 t + \beta_2 t^2 + \beta_3 t^3 \quad [1]$$

Selected pairs of lines were compared utilizing the principle of conditional error (Swindel 1970). The difference in error sums of squares between a full and two reduced models were compared using an F test. The full model included the data common to the two groups being compared while the two reduced models included only the data from each individual group. The test statistic for this procedure is given by:

$$F = \frac{(SSE^* - SSE) / (f^* - f)}{SSE / f} \quad [2]$$

where SSE^* is the error sums of squares for the full or common regression; f^* is the error degrees of freedom for the full or common regression; SSE is the pooled error sums of squares for the reduced models; and f is the pooled error degrees of freedom of the reduced models. This test

statistic is compared with an F value with (f^*-f) degrees of freedom in the numerator and f degrees of freedom in the denominator. This is essentially a multivariate procedure that tests for differences in the overall regression rather than differences between individual pairs of data points.

Phosphorus desorption data were fitted to the cubic equation using the REG procedure of the Statistical Analysis system (SAS). Computing was done using the facilities of the Northeast Regional Data Center of the State University System of Florida, located on the campus of the University of Florida in Gainesville.

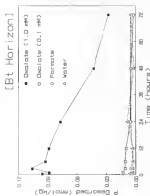
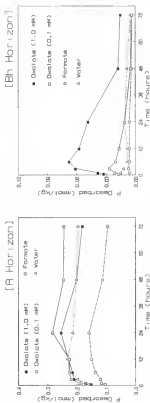
Results

Inorganic P (fig. 3-1)

Statistical comparisons of the desorption curves indicated that for the A horizon the differences among 1.0 mM oxalate, 0.1 mM oxalate and water were not significant. In the Bt horizon, inorganic P desorption in formate was not different from that in water. All other comparisons were statistically significant ($p < 0.05$).

As pointed out above, the release of inorganic P in the water and oxalate solutions was similar in the A horizon. In the A horizon soil material, inorganic P desorption increased rapidly for the first 6 to 12 hours.

Figure 3-1. Kinetics of inorganic P desorption into 0.1 mM and 1.0 mM oxalate, 1.0 mM formate and distilled water adjusted to pH 4.3 for A, Bh and Bt horizon material from a Pomona series soil. (Note differences in ordinal scale).



After 24 hours inorganic P declined slightly. Phosphorus desorption in formate was considerably lower. Inorganic P desorption in formate was greater, however, in the A horizon than in the Bh and Bt horizons.

In the Bh horizon, release of inorganic P was greater in the oxalate solutions than in water. Between 2 and 3 times more inorganic P was desorbed in the 1.0 mM oxalate compared to the 0.1 mM oxalate. In both oxalate solutions, inorganic P desorption increased rapidly for 3 to 6 hours and then immediately decreased again. The point where P desorption began to decline coincides with the complete sorption of the added oxalate (fig. 3-3b). Phosphorus desorption in water declined monotonically during the first 6 hours. Inorganic P desorption in the formate solution was less than water.

The general pattern of P desorption in the Bt horizon was similar to that in the Bh horizon. There was a short period where P release increased in the 1.0 mM oxalate solution which continued until all the added oxalate was sorbed (fig. 3-3c). In the Bt horizon, the 1.0 mM oxalate solution caused a 9-10 fold increase in P desorption. Phosphorus desorption in the 0.1 mM oxalate was slightly greater than in water or formate. In these latter two solutions, P desorption was negligible.

Organic P (fig. 3-2)

Appreciable amounts of organic P were released in the A and the Bh horizons but not in the Bt horizon. Organic P comprised some 10 to 30 % of the total P released in the A horizon and 30 to 70 % in the Bh horizon. In the Bt horizon, however, the proportion and absolute amount of organic P were small. The overall pattern of organic P desorption in the various solutions was similar in the A and Bh horizons. Desorption of organic P increased gradually and then leveled off after 6 to 12 hours. Initially, organic P desorption in both oxalate solutions was greater than water and the 1.0 mM solution was greater than the 0.1 mM solution. Organic P desorption in the formate was less than water in both horizons. In the A horizon, release of organic P by the two oxalate solutions decreased sharply after 24 hours, whereas the decrease in organic P was small and gradual in the rest of the solutions.

Oxalate Sorption (fig. 3-3)

Approximately 1mM/kg of oxalate was sorbed in the first 12 hours from both oxalate solutions in the A horizon. This represents all the added oxalate in the 0.1 mM solution. Formate was not sorbed at all during this same period. After 12 hours, the remaining oxalate and formate were rapidly degraded. The 10 mM/kg value represents all the added organic acid in the 1.0 mM solutions. The term "degradation" is used to describe the abrupt drop in

Figure 3-2. Kinetics of organic P desorption into 0.1 mM and 1.0 mM oxalate, 1.0 mM formate and distilled water adjusted to pH 4.3 for A, Bh and Bt horizon material from a Pomona series soil. (Note differences in ordinal scale).

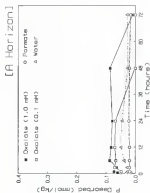
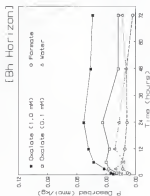
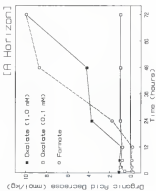
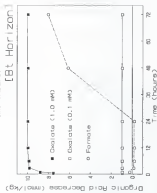
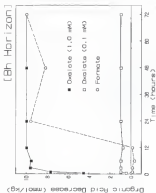


Figure 3-3. Kinetics of disappearance from solution of 0.1 mM and 1.0 mM oxalate and 1.0 mM formate added to A, Bh and Bt horizon material from a Pomona series soil.



solution concentrations of organic acids after 24 hours, since it is unlikely that this is an adsorption reaction. Support for this conclusion will be presented in the discussion section. In the two subsurface horizons, all the added oxalate disappeared within 12 hours. None of the added formate disappeared during this same time. After 12 to 24 hours, however, the formate disappeared rapidly.

Aluminum (fig. 3-4)

Among the three horizons, Al release increased in the order Bh horizon > Bt horizon > A horizon. The overall pattern of release was, however, the same in each horizon. Release of Al in water was consistently small. The release of Al in 1.0 mM oxalate solution was greater than in 0.1 mM oxalate or formate. In each of the organic acid solutions, there was a short period of rapid Al release lasting approximately 3 hours. In the 1.0 mM oxalate solution, this was followed by an extended period of gradual Al release. The Al levels in the formate solution began to decline after 12 to 24 hours. The Al levels in the oxalate solutions also declined, but for the most part not until after 48 hours.

Solution pH (fig. 3-5)

In the A horizon, the pH of the oxalate solutions and water were initially similar while the pH of the formate solution was lower. All were stable through 12 to 24 hours, after which the pH of the organic acid solutions rose

Figure 3-4. Kinetics of Al release into 0.1 mM and 1.0 mM oxalate, 1.0 mM formate and distilled water adjusted to pH 4.3 for A, Bh and Bt horizon material from a Pomona series soil. (Note differences in ordinal scale).

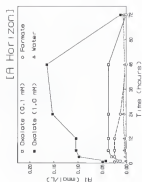
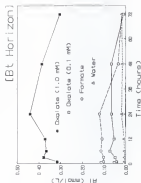
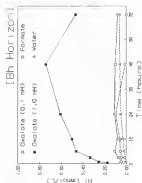
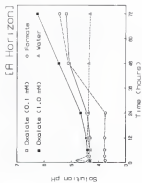
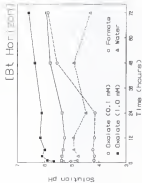
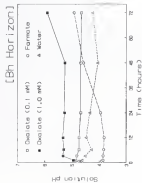


Figure 3-5. Temporal changes in solution pH of 0.1 mM and 1.0 mM oxalate, 1.0 mM formate and distilled water initially adjusted to pH 4.3 extracts from A, Bh and Bt horizons from a Pomona series soil



sharply corresponding with the disappearance of the organic acids. In the subsoil, the pH of the oxalate solutions was greater than water and tended to increase slowly. There was a gradual decrease in pH with time in the water extract. As in the A horizon, the pH of the formate solution was lower than water until 24 hours. At the point where the formate began to degrade, the pH of the solution again rose sharply.

Discussion

The foregoing results indicate that the mechanism controlling inorganic P release in the A horizon differed from that in the Bh and Bt horizons. In light of this, the surface and subsurface soils will be discussed separately. The discussion is based on the relative ability of the solutions to complex Al (oxalate > formate \approx water) and the hypothesis that if P reactions are controlled by Al, the amount and rate of P desorption should follow the same progression.

A Horizon

The similarity of P release among the oxalate solutions and water during the first 12 hours suggests that ligand-exchange reactions at Al surfaces were not controlling P release in the A horizon. The organic acid sorption data support this conclusion. Although P desorption in the formate solution was less than in water, relatively large amounts of P were still released in the formate solution. No

formate was sorbed, however, during the initial 12-hour period. In addition, even though a limited amount of oxalate was sorbed by 12 hours, P release was no greater than in water. These results preclude ligand exchange as the mechanism of P release in the A horizon. At its natural pH, the A horizon was essentially devoid of surfaces that react with P. The P that was present was apparently in a water-soluble form. Previous work with surface horizons soil material from flatwoods Spodosols has shown that they have almost no ability to sorb added P and that P fertilizers are rapidly leached from them (Humphreys and Pritchett 1971; Ballard and Fiskell 1974).

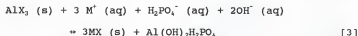
Because small amounts of exchangeable Al were present in the A horizon (Table 3-1), the ability of organic acids to complex Al did affect P release. In the water extracts very little Al was released and the effect on solution P was slight. Al release was greater in the formate solution because of its higher ionic strength. The Al would hydrolyze and polymerize freely in the formate solution because formate does not form stable complexes that retard these reactions (Huang and Violante 1986). The drop in pH of the formate solution supports this interpretation. Reactions between P and Al hydrolysis products probably caused the decrease in net P desorption observed in the formate solution. Although the formation of stable complexes in the oxalate solution probably stimulated the release of Al, its

hydrolysis and polymerization would have been inhibited by chelating ligands in solution (Huang and Violante 1986). Therefore, although the amount of Al in the oxalate solutions was greater, the stable complexes probably hindered the formation of solid phase Al phosphates (Ng Kee Kwong and Huang 1977).

The rapid drop in oxalate and formate concentrations after 12 hours was probably caused by degradation of the organic acids rather than adsorption. McColl et. al. (In press) have observed rapid degradation of organic acids in soil solution, which they attributed to both biotic and abiotic pathways. Simple organic acids may serve as the substrates for growth of microorganisms (Harder 1973; Hodgkinson 1977). Although toluene was added to retard microbial growth in the samples of this study, microbial activity was probably only inhibited for a short while. O'Keefe et al. (1987) observed significant activity of both bacteria and fungi after 24 hours in similar soils treated with toluene. Hodgkinson (1977) summarized a variety of chemical degradation pathways for both oxalate and formate. Jauregui and Reisenauer (1982) have outlined a reaction scheme whereby organic acids, including oxalate and formate, are oxidized as Mn-oxides are reduced in soil. Chemical oxidation of oxalate may also be catalyzed by Al (Hodgkinson 1977). The oxidation of organic acids tends to consume hydrogen ions and would cause the solution pH to rise.

The gradual drop in the concentration of inorganic P after 24 hours was interpreted as the formation of colloidal Al-phosphates. Chemical speciation with the computer program GEOCHEM (Sposito and Mattigod 1980) was used to explore these possibilities. Results from the GEOCHEM program must be viewed with caution since it assumes equilibrium conditions, an obviously untenable assumption in the present study. Calculated ion-activity-products (IAP) showed there was a tendency for the solutions to become saturated with respect to amorphous $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$. For example, in the oxalate solutions the log values of the IAP for $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$ were stable at 28 to 29 between 6 and 24 hours suggesting undersaturation with this solid phase which has a $\log K_{sp}$ value of 27 to 29 (Coleman et al. 1960; Taylor and Gurney 1962; Veith and Sposito 1977). After 24 hours, the organic acids began to degrade and the solution pH increased. At 48 hours the calculated log IAP for $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$ dropped to approximately 26, suggesting that the solution was now saturated with respect to this solid phase. It was at this time that the levels of P in solution began to drop, probably in association with the formation of a colloidal $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$ solid. The formation of amorphous $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$ has been reported for a variety of acid soils (Coleman et al. 1960; Taylor and Gurney 1962; Chen et al. 1973a, 1973b; Veith and Sposito 1977; Traina et al. 1986a). The above sequence of events is similar to that proposed by Coleman et

al. (1960) for the reactions between Al-montmorillonite and P:



where X represents the exchanger surface and M^+ an exchangeable cation. This equation suggests that in the presence of exchangeable Al and an aqueous metal cation, solution P levels will drop with increasing pH, or increasing background electrolyte concentration (Traina et al. 1986a). This was exactly what happened in the A horizon soil in this study.

Bh and Bt Horizons

The Bh and Bt horizons both contain surfaces dominated by Al that specifically sorb large amounts of P (Table 3-1) (Yuan 1966; Humphreys and Pritchett 1971). Since P release was greater in the more concentrated oxalate solution, and low in both formate and water, the ability to form stable complexes with Al was important for the release of P from the subsoil horizons where P is fixed by Al. Numerous studies have reported similar results (Kurtz et al. 1946; Swenson et al. 1949; Low and Black 1950; Deb and Datta 1967; Appelt et al. 1975a, 1975b; Lopez-Hernandez et al. 1979; Comerford and Skinner 1989).

The rapid sorption of oxalate, which was accompanied by rapid release of P and an increase in solution pH, suggested ligand exchange was the mechanism responsible for P release

in the Bh and Bt horizons. Ligand exchange reactions at metal-oxide surfaces are rapid and tend to release OH^- (Goldberg and Sposito 1985). Another observation supporting the ligand-exchange hypothesis was the fact that P desorption in the oxalate solutions continued only as long as oxalate sorption continued.

Sorption of oxalate was much greater in the Bh and Bt horizons than in the A horizon because of the presence of reactive Al surfaces. Since all the oxalate was sorbed within 12 hours in both subsurface horizons, the rapid pH increase that accompanied oxalate degradation in the A horizon did not occur. As in the A horizon, none of the added formate was sorbed initially; however, after 12 to 24 hours the remaining formate was quickly degraded which was accompanied by a rapid rise in solution pH.

As in the A horizon, oxalate in solution was able to complex Al and inhibit its hydrolysis and polymerization. GEOCHEM calculations indicated that over 95 % of the Al in solution was complexed with oxalate during the first 6 hours. After the added oxalate was sorbed, however, the formation of colloidal Al-phosphates was probably responsible for the observed drop in solution P levels in the Bh and Bt horizons. For example, the calculated log IAP of $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$ was approximately 26 in the Bh horizon at 12 hours suggesting that this solid phase may have been forming.

As in the A horizon, very little Al was released in water. Hydrolysis of the small amounts of Al released, however, was probably responsible for the gradual drop in pH and P levels in the water extracts. The data in Table 3-1 suggest that both exchangeable and solid-phase Al were present in the Bh and Bt horizons. The initial rapid release of Al in the organic acid solutions was probably associated with release of exchangeable Al since it occurred in formate as well as oxalate. The lower pH of the formate solutions suggests that the exchanged Al hydrolyzed and polymerized. Reactions between P and Al polymers also probably decreased solution P levels in the formate samples. The differences in P desorption between water and formate were small, however, since very little P was initially released in either solution. The gradual release of Al in the oxalate solutions beyond 6 to 12 hours was attributed to the dissolution of solid-phase Al surfaces. Dissolution reactions tend to be much slower than exchange reactions (Sparks 1986; Stumm 1986). It was interesting that Al release continued even after all the oxalate was sorbed. This result conforms to the theory of metal-oxide dissolution proposed by Stumm (1986). He suggests that surface complexation by ligand exchange is more important in the dissolution of Al-oxide surfaces than metal complexation in solution. According to Stumm (1986), ligand exchange at metal-oxide surfaces decreases the strength of the remaining

metal-OH-metal bonds and the surface dissolves. This mechanism may explain the gradual release of Al in the Bh and Bt horizons.

Organic Phosphorus

Soluble organic P is seldom knowingly included in studies of soil P. The large amount of organic P released in the A and Bh horizons of this soil was interesting. In both these horizons, the release of organic P was greater in the oxalate solutions than in the water or formate solutions. This was interesting in light of the lack of a similar relationship for inorganic P in the A horizon. Another interesting difference between organic P and inorganic P was the lack of substantial resorption of organic P in the Bh horizon.

Certain forms of organic P, such as the inositol phosphates, are sorbed to soil surfaces in a manner similar to inorganic P (Wild and Oke 1966; Martin 1970; Anderson et al. 1974). Thus the ability of oxalate to complex metals, such as Al, may affect organic P release. Other organic P compounds, however, may be retained in a different manner (Kowalenko 1978). Since the forms of organic P in these soils is unknown, the mechanism by which oxalate influences organic P is uncertain. Additional work is needed to identify the forms of organic P in these soils and the influence of organic acids on their solubility. The presence of large amounts of soluble organic P, between 10

and 30 % of the total pool of P released in the A horizon, and up to 70 % of that released from the Bh horizon, has important implications for availability of P to plants growing in these Spodosols.

Implications for Tree Nutrition

Phosphorus availability often limits growth of pine trees planted in Spodosols of the flatwoods (Pritchett and Comerford 1983). The results of this study suggest that oxalate increases the pool of labile P in the subsoil. This may have a large impact on P uptake from these soils.

One way to estimate the potential impact of oxalate on P uptake of pine trees is through the use of mechanistic nutrient-uptake models such as the ones developed by Nye and Tinker (1977) and by Barber (1984). Sensitivity analyses with these models suggest that three soil parameters have a large impact on P uptake: the initial concentration of P in solution (C_{11}); the P buffer power of the soil (b); and the effective diffusion coefficient of P in soil (D_e). Increasing each of these parameters increases P uptake.

The increase in P release in the subsoil when oxalate was present can be directly translated into an increase in C_{11} . As will be shown in Chapter 6, oxalate tends to increase the buffer power of the subsoil. The effect of oxalate on D_e is more complicated. The effective diffusion coefficient in soil is defined as

$$D_e = D_1 \theta f / b \quad [4]$$

where D_1 is the diffusion coefficient in water; θ is the water content; f is the tortuosity factor; and b is the buffer power. It can be seen in Eq. 4 that increasing b decreases D_1 , but the relative effect of oxalate on these opposing factors in relation to P uptake needs to be investigated. Incorporation of the effects of oxalate into mechanistic models would be a valuable and informative exercise.

Van Rees and Comerford (1986) have shown that fine roots of slash pine tend to proliferate in the argillic horizon of Spodosols. Dense root mats also are frequently observed at the surface of spodic horizons. Comerford et al. (1984) and Neary et al. (In press) have shown through the use of a mechanistic nutrient-uptake model (Nye and Tinker 1977), that the subsurface horizons may contribute a substantial portion of the P taken up by trees. Their work did not, however, incorporate the increased P availability that might be due to oxalate. Since oxalate had no effect in the A horizon, but substantially increased P availability in the subsoil, incorporating the effects of oxalate on P release should significantly increase the relative importance of subsurface horizons. The implications of this for tree nutrition are important, especially in light of the results from Chapter 2 which demonstrated that higher oxalate concentrations occur in the subsoil. Phosphorus

uptake from subsoils has the potential to be an important factor determining forest productivity in the flatwoods.

The estimates of nutrient uptake by Neary et al. (In press) also did not include the potential contribution of soluble organic P. The presence of phosphatase enzymes in soil suggest that soluble organic P may be a relatively available form of P (Speir and Ross 1978) and should be included in the pool of plant labile P in these soils.

Conclusions

Of the two LMW organic acids commonly found in soil solutions of poorly-drained Spodosols, only oxalate was able to stimulate release of inorganic P. This effect was observed in the Bh and Bt horizons. Apparently there were no Al-oxide surfaces in the A horizon capable of reacting with inorganic P. The concentrations of P observed will depend on the interaction between ionic strength of the extracting solution and presence of complexing agents. Solution P levels will tend to drop when exchangeable Al is released due to the formation of amorphous $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$. Complexing agents tend to inhibit this reaction by reducing the amount of free Al available. Al-hydroxyl surfaces in the Bh and Bt horizons apparently hold P as an inner-sphere complex. Oxalate is able to release this sorbed P by a ligand-exchange reaction. As long as oxalate was present in solution, the formation of colloidal Al-P was inhibited. In

the absence of oxalate, colloidal Al-P solids tend to form and reduce the P levels in solution. Calculated IAP values suggest that $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$ was the solid-phase phosphate formed. Oxalate also increased organic P release in both the A and Bh horizons. The lack of information on the form of organic P released prevents any definitive conclusions concerning the mechanisms of its release.

The results from this study suggest several areas that require further investigation. First is the relationship between the ability of organic acids to complex Al and thereby release P from subsoil horizons. Since the suite of organic acids present in soils is quite variable, it would be valuable to have a way of estimating P release when different organic acids are present. The Al stability constant ($\log K_{\text{Al}}$) might be a useful property of organic acids in this regard. Second, since production of organic acids tends to occur at point sources in the soil, such as a root or fungal hyphae, the actual oxalate concentration at the soil surface is unknown. The data from soil extracts and even displaced soil solutions such as those in Chapter 2 invariably reflect some degree of dilution of the organic acid concentration. The results in this chapter have shown a large effect of increasing oxalate concentration on P desorption. Therefore, to bracket the possible loading rates to which a soil could be exposed, it would be valuable to examine a wide range of organic acid loading rates.

Finally, effects of a single oxalate addition on P desorption in these soils were ephemeral. Organic acids are produced more-or-less continuously in soils. Therefore, it would also be valuable to examine the effects of repeated additions of organic acids on P release. These three areas are investigated in the following chapters.

CHAPTER 4
PHOSPHORUS AND ALUMINUM RELEASE FROM SPODIC HORIZON
MATERIAL IN THE PRESENCE OF LOW-MOLECULAR-WEIGHT ORGANIC ACIDS

Introduction

Low-molecular-weight (LMW) organic acids in soils function as organic ligands and interact with metals and metal hydroxide surfaces through complexation reactions (Stumm 1986; Martell et al. 1988). The ability of organic acids to directly influence solution and surface reactions of P and Al in soils is primarily related to their ability to form stable complexes with Al. The occurrence and position of carboxylic and phenolic functional groups on organic acids determines their ability to complex Al (Schnitzer and Skinner 1985; Martell et al. 1988). Stable complexes tend to contain five- and six-membered rings involving these functional groups and a central Al cation (Cotton et al. 1987). The size of the Al-organic acid stability constant ($\log K_{Al}$) serves as a measure of the strength of the complex. Therefore, it should be possible to estimate the relative effects of organic acids on P and Al release in soils based on the $\log K_{Al}$ value of the respective Al complexes.

In Chapter 3, the release of Al and P from a Florida Spodosol (Ultic Haplaquod) was investigated in the presence of oxalate and formate. Larger amounts of both Al and P were released in a 10^{-3} M oxalate solution compared to a 10^{-3} M formate solution. The difference was attributed to the greater Al stability constant of oxalate ($\log K_{Al} = 6.10$) compared to formate ($\log K_{Al} = 1.36$). Traina et al. (1987) showed a similar trend with greater release of P, Al, Si, and Ca from an acid montmorillonitic soil in the presence of citrate ($\log K_{Al} = 7.98$) compared with formate. Several studies with forest soils from California have shown greater release of Al and other metals in the presence of organic acids with larger $\log K$ values (McColl and Pohlman 1986; Pohlman and McColl 1986). These authors noted that in some cases, however, very little metal dissolution occurred in the presence of organic acids with relatively large $\log K$ values. In the classic study by Low and Black (1950), P release from soil was generally greater when complex forming organic acids were added. Based on recent $\log K_{Al}$ values of the organic acids used, however, their data also shows some irregularities in P release. Hue et al. (1986) related the ability of organic acids to alleviate Al toxicity in cotton plants with their ability to complex Al, since Al in complexed form is generally not toxic to plants. They also noted some inconsistencies in this relationship when it was based on published $\log K_{Al}$ values. In the above studies,

salicylic acid always behaved anomalously. In spite of a large Al stability constant ($\log K_{Al} = 12.9$), it had little effect on the reactions studied.

The above results led McColl and Pohlman (1986) to conclude that metal stability constants ($\log K$) were not a good predictor of the ability of an organic acid to solubilize metals in forest soils. Basic principles of coordination and surface chemistry, however, suggest that if the mechanisms responsible for P release and Al dissolution are ligand exchange and the formation of inner-sphere complexes at Al-hydroxide surfaces, stability constants should be related to P release and Al dissolution. The results in Chapter 3 indicate that ligand exchange was in fact the dominant mechanism of P and Al release from a spodic horizon dominated by Al. Therefore, the purpose of this chapter was to examine the relationship between the $\log K_{Al}$ value of LMW organic acids and P and Al release from a representative soil material from a spodic horizon. A variety of organic acids were used in this experiment to give a range of $\log K_{Al}$ values with which to test the relationship.

Materials and Methods

Soil Material

Spodic (Bh) horizon soil material from a Pomona series (sandy, siliceous, hyperthermic Ultic Haplaquod) was

collected from a single soil pit located in Alachua County, Florida. The soil material was air-dried, passed through a 2-mm sieve, and stored in a plastic barrel prior to use. This soil was also studied in Chapter 3 and selected chemical and physical properties, determined by standard methods (Page et al. 1982; Klute 1984) are presented in Table 3-1.

Organic Acid Extractions

Sixteen organic acids, including both aliphatic and aromatic compounds, that have been identified as natural constituents of soil solutions (Stevenson 1967) were studied in this experiment (Table 4-1). Distilled water was also included as a reference. Millimolar solutions of the organic acids were prepared from reagent-grade material. The solutions were adjusted to pH 4.3 with HCl or NaOH. No attempt was made to control ionic strength of the solutions, which ranged from 0.4 mM in water to 8.2 mM in vanillic acid.

The published $\log K_{A1}$ of the organic acids were adjusted to 0.1 M ionic strength following the procedure suggested by Martell et al. (1988). This procedure is based on an empirical tabulation of changes in stability constants with changes in ionic strength.

Duplicate, 10-g samples of air-dried soil material were placed in 125-mL polyethylene bottles and 50 mL of an organic acid solution or water were added along with two

Table 4-1. Properties of low-molecular-weight organic acids used to extract P and Al.

Organic Acid	Form	LogK _{Al}	Ref	Adj ⁴ LogK _{Al}
Acetic CH ₃ CO ₂ H	HL	1.57(1.0) ¹	a ²	1.60
Aconitic HO ₂ CCH=C(CO ₂ H)CH ₂ CO ₂ H	H ₃ L	- ³		
Citric HO ₂ CCH ₂ C(OH)(CO ₂ H)CH ₂ CO ₂ H	H ₃ L	7.98(0.1)	b	7.98
Formic HCO ₂ H	HL	1.36(0.1)	a	1.36
Fumaric HO ₂ CCH=CHCO ₂ H	H ₂ L	-		
p-Hydroxybenzoic HOC ₆ H ₄ CO ₂ H	H ₂ L	1.66(0.1)	f	1.66
Lactic CH ₃ CH(OH)CO ₂ H	HL	2.38(0.2)	c	2.41
Maleic HO ₂ CCH=CHCO ₂ H	H ₂ L	-		
Malic HO ₂ CCH ₂ CH(OH)CO ₂ H	H ₂ L	5.34(0.2)	c	5.40
Malonic HO ₂ CCH ₂ CO ₂ H	H ₂ L	5.24(0.2)	e	5.30
Oxalic HO ₂ CCO ₂ H	H ₂ L	6.10(1.0)	a, d	6.16
Pectic [(CO ₂ H)C ₅ (OH) ₅ H ₄ O] _x	H _x L	-		

Table 4-1. Continued.

Organic Acid	Form	LogK _{A1}	Ref	Adj LogK _{A1}
Phthalic C ₆ H ₆ -1,2 (CO ₂ H) ₂	H ₂ L	3.18(0.1)	c	3.18
Salicylic HOC ₆ H ₄ CO ₂ H	H ₂ L	12.9(0.1)	a	12.9
Succinic HO ₂ CCH ₂ CH ₂ CO ₂ H	H ₂ L	2.09(1.0)	g	2.15
Tartaric HO ₂ CCH(OH)CH(OH)CO ₂ H	H ₂ L	5.62(0.1)	a	5.62
Vanillic HOC ₆ H ₃ (OCH ₃)CO ₂ H	H ₂ L	-		

1. Literature logK values listed are for 1:1 organo-metal complexes. The ionic strength is in parentheses.
2. References for published logK_{A1} values:
 - a) Martell and Smith 1977;
 - b) Motekaites and Martell 1984;
 - c) Perrin 1979;
 - d) Martell and Smith 1982;
 - e) Sillen and Martell 1971;
 - f) Pohlman and McColl 1986;
 - g) Christensen and Izatt 1983;
3. No published value available.
4. LogK value from the literature adjusted to 0.1 M ionic strength following the procedure of Martell et al. (1988).

drops of toluene. The samples were shaken on a reciprocating shaker at 100 cycles per minute for 6 hours. Previous work with this soil in Chapter 3 indicated that P desorption reaches a maximum after 3 to 6 hours. After shaking, the samples were filtered through 0.45 micron nylon membrane filters.

The conductivity of the filtrate was measured with a platinum conductivity electrode on an YSI conductivity meter. Solution pH was measured with a combination glass electrode on an Orion pH meter. Aluminum was determined using flame emission spectrophotometry with a $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame.

Inorganic P in the filtrate was determined by a molybdenum-blue colorimetric procedure using ascorbic acid as a reductant (Murphy and Riley 1962). This is an operational definition of inorganic P, since the molybdenum hydrolyzes some organic P compounds (Stainton 1980; Tarapchak et al. 1982). Total P was also measured in each sample as follows: A 20 mL aliquot of the filtrate was evaporated to dryness at 100 °C in a 50-mL pyrex beaker. The beaker was then placed in a muffle furnace overnight at 500 °C. The beaker was allowed to cool and then placed on a hot plate. A 10-mL aliquot of 40% HCl was added to each sample and evaporated to dryness followed by a 5-mL aliquot of concentrated HCl which was also evaporated to dryness. The beakers were allowed to cool and the sample was redissolved in 20 mL of 0.1 N HCl. Phosphorus in solution was again

measured by the Murphy-Riley procedure. Soluble organic P was operationally defined as the difference between Murphy-Riley P in the dry-ashed and acid-digested sample (total P) and Murphy-Riley P in the initial filtrate (inorganic P).

Statistical Analysis

Analysis of variance (ANOVA) was used to test for differences in P and Al release among the various organic acids. Mean separations were performed using Duncan's multiple range test (Gomez and Gomez 1984).

The relationship between $\log K_{Al}$ and P or Al release was examined using regression analysis. The data was first fit to a linearized form of the exponential equation:

$$\ln Al \text{ or } \ln P = \ln \alpha + \beta(\log K_{Al}). \quad [1]$$

Where 2 straight line segments seemed more appropriate, they were also fit to the data using simple linear regression. $\log K_{Al}$ values for those organic acids without published literature values were predicted utilizing the exponential relationship between P release and $\log K_{Al}$ obtained above.

The ANOVA and Duncan's multiple range tests were performed using the GLM procedure of the Statistical Analysis System System (SAS). Regression analyses were performed using the REG procedure. Computing was done using the facilities of the Northeast Regional Data Center of the State University System of Florida, located on the campus of the University of Florida in Gainesville.

Results

Release of P and Al in the various organic acid solutions is presented in Table 4-2. The release of inorganic P varied by over an order of magnitude among the various organic acids. In 11 of the organic acid extracts, release of inorganic P was no different than in water. In each of these extracts, where known, the $\log K_{Al}$ was always below 4. The amounts of inorganic P released were greater in the organic acids with larger $\log K_{Al}$ values. Salicylic acid was however, a notable exception to this trend. It had the largest $\log K_{Al}$ and yet relatively small amounts of Al and inorganic P were released. Except for tartarate, malate, oxalate and citrate, release of organic P was greater than inorganic P. Release of Al was 1 to 2 orders of magnitude greater than release of P in this soil, however, the trends in Al release were generally the same as with inorganic P.

The relationship between Al release and the $\log K_{Al}$ values for the 11 organic acids with published $\log K_{Al}$ values is presented in figure 4-1. Because of its anomalous behavior, salicylic acid was not included in the calculated relationship. The release of Al increased exponentially with the $\log K_{Al}$ value. The coefficient of multiple determination for the linearized relationship was relatively high ($R^2 = 0.704$). The same general relationship was observed between release of inorganic P and $\log K_{Al}$ values (fig. 4-2).

Table 4-2. Phosphorus and Al release from Pomona Bh horizon material in 1.0 mM organic acid solutions at a 5:1 solution/soil ratio.

Organic Acid	Adj LogK _{a1}	Ionic Strength (mM)	Al	Inorganic P	Organic P	Organic P	Calc ² LogK _{a1}
			----- (mmol/kg) -----			(%)	
Lactic	2.41	2.5	1.251 d ³	0.002 a	0.009 a	82.3	0.80
Formic	1.36	2.7	0.994 c	0.002 a	0.018 abc	86.6	1.78
Maleic		1.9	0.321 a	0.003 a	0.011 ab	79.0	1.93
Phthalic	3.18	1.5	0.321 a	0.003 a	0.009 a	75.7	2.01
Acetic	1.60	1.7	0.192 a	0.003 a	0.010 ab	76.5	2.09
Succinic	2.15	1.1	0.192 a	0.003 a	0.010 ab	74.9	2.31
Vanillic		8.2	0.321 a	0.004 a	0.020 abc	84.9	2.40
Water		0.4	0.192 a	0.004 a	0.012 ab	76.8	
p-Hydroxybenzoic	1.66	0.8	0.257 a	0.004 a	0.013 ab	75.7	2.67
Aconitic		1.8	0.289 a	0.004 a	0.011 ab	72.6	2.68
Fumaric		2.0	0.289 a	0.004 a	0.022 abc	82.5	2.95
Salicylic	12.9	1.4	0.577 b	0.005 a	0.012 ab	70.7	3.21
Malic	5.40	1.7	1.219 d	0.012 b	0.025 bc	70.2	4.90

Table 4-2. Continued.

Organic Acid	Adj LogK _{Al}	Ionic Strength	Al	Inorganic P	Organic P	Calc LogK _{Al}
		(mM)	-----	(mmol/kg)	-----	(%)
Tartaric	5.62	2.4	1.892 e	0.013 bc	0.007 a	36.4
Malonic	5.30	2.5	2.406 f	0.015 c	0.012 ab	42.0
Oxalic	6.16	2.0	2.790 g	0.035 d	0.029 c	45.3
Citric	7.98	3.9	6.254 h	0.039 e	0.014 abc	25.7
						7.80

1. LogK values were adjusted to 0.1 M ionic strength following the procedure of Martell et al. (1988).

2. Calculated logK values were obtained from the relationship between logK and inorganic P release in figure 4-2.

3. Values within a column with the same letter are not significantly different according to Duncan's multiple range test ($\alpha = 0.05$).

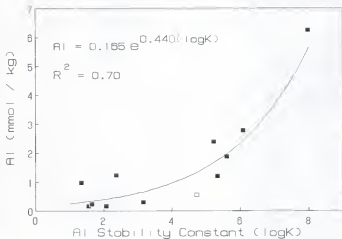


Figure 4-1. Relationship between Al release and $\log K_{Al}$ values of organic acids in Pomona Bh horizon material. The open square represents the estimated $\log K_{Al}$ value of salicylic acid from Hue et al. 1986.

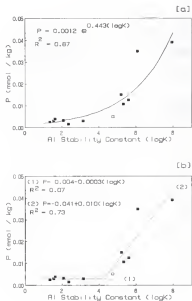


Figure 4-2. Relationship between inorganic P release and $\log K_{Al}$ value of organic acids in Pomona Bh horizon material: [a] Exponential fit to the data; [b] Two straight-line segments fit to the data. Open square represents the estimated $\log K_{Al}$ value for salicylic acid from Hue et al. 1986.

Excluding salicylate again, the exponential relationship was much more consistent as reflected in the higher R^2 value (0.865). The relationship between inorganic P release and $\log K_{Al}$ values suggests that a threshold value for $\log K_{Al}$ exists, below which inorganic P is not affected. This threshold $\log K_{Al}$ value appears to be between 4 and 5. By fitting two straight-line-segments to the data in figure 4-2, the threshold $\log K_{Al}$ value, calculated as the point of intersection between the two lines, was 4.14.

In marked contrast, the release of organic P was fairly uniform and essentially independent of the ability of the organic acids to complex Al (fig. 4-3). The three points above the bulk of the data are for formate, malate and oxalate.

Release of inorganic P was well correlated with Al release in this soil (fig. 4-4) with a simple correlation coefficient of 0.909. In contrast, the correlation coefficient between organic P and Al was only 0.142 (data not presented).

Discussion

Because P is retained as an inner sphere complex at Al-hydroxide surfaces and released via a ligand-exchange reaction (see Chapter 3; Parfitt et al. 1977; Sposito 1984),

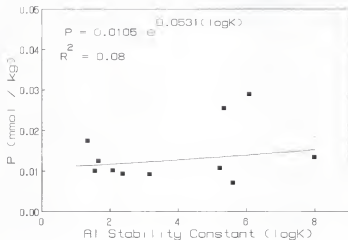


Figure 4-3. Relationship between organic P release and $\log K_{Al}$ of organic acids in Pomona Bh horizon soil material.

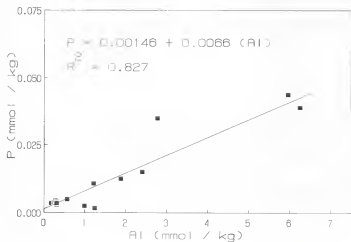


Figure 4-4. Relationship between inorganic P and Al release in Pomona Bh horizon soil material.

there was a strong relationship between inorganic P release and the $\log K_{Al}$ value of the organic acids. The ligand exchange mechanism was confirmed for the soil used in this study in Chapter 3. With the obvious exception of salicylic acid, the $\log K_{Al}$ value seems to reflect the ability of the organic acids to enter into ligand exchange reactions and replace P at Al-hydroxide surfaces in the spodic horizon. These data (fig.4-2) indicated that a threshold $\log K_{Al}$ value of between 4 and 5 was required before substantial amounts of P were released. This suggests that the stability constant for P complexes at Al-hydroxide surfaces would be in this range. Organic acids with a smaller $\log K_{Al}$ form less stable surface complexes than P and were not able to replace P at Al-hydroxide surfaces. According to Hingston et al. (1968, 1972) the ability of a competitor to replace a specifically adsorbed anion depends on its ability to increase the negative charge on the surface. The $\log K_{Al}$ value of 4.14 may be related to this phenomena and the ability of organic acids to alter the charge on the oxide surface.

A number of LMW organic acids have been isolated from forest soils which may have an impact on cycling and availability of nutrients, such as P. The results from this study suggest that it may be possible to estimate the relative effect of an organic acid on P availability based on its ability to complex metals. However, the $\log K$ values

measured in solution by standard methods may not accurately reflect the ability of the ligand to form surface complexes, as shown by salicylate. The release of both Al and P was anomalous in the salicylic acid extract. This same result was observed by Low and Black (1950), Pohlman and McColl (1986) and Hue et al. (1986). Hue et al. (1986) used a novel approach to estimate $\log K_{Al}$ for organic acids based on their ability to alleviate symptoms of Al toxicity in cotton roots. From this relationship, the effective $\log K_{Al}$ value of salicylic acid was estimated to be 4.74. The open square in figs. 4-1 and 4-2 is the point where salicylate would occur utilizing the calculated $\log K_{Al}$ of Hue et al. (1986). This estimated $\log K$ conforms well to observed amount of P released by salicylate in the soil used in our study. It would also fit the data of Low and Black (1950). Apparently, salicylic acid is unable to form stable surface complexes as well as indicated by its $\log K_{Al}$ value calculated in solution. Because P release in most soils occurs via a ligand exchange reaction at surfaces, additional work on determining the stability of surface complexes of naturally occurring organic acids is needed.

The success of Hue et al. (1986) in predicting an "effective" $\log K_{Al}$ for salicylic acid was interesting. This suggests that the relationship in fig. 4-3 could also be used as a way of estimating "effective" $\log K_{Al}$ for organic acids with unknown values since P release is controlled by

ligand exchange. Estimates of $\log K_{Al}$ back-calculated in this manner based on P release are included in Table 4-2.

Organic acids with large $\log K_{Al}$ value would affect Al release from Al-hydroxide surfaces which dissolve according to the mechanisms suggested earlier by Stumm (1986). These mechanisms all involve the formation of stable complexes and thus depend on the $\log K_{Al}$ value of the organic acid present. However, both exchangeable Al and Al-hydroxide surfaces were present in this soil material. The $CaCl_2$ and KCl extractable Al presented in Table 3-1 gives the order of magnitude of the exchangeable Al. Exchangeable Al would be released by a simple cation exchange reaction. Organic acids that form stable complexes with Al would only have a secondary effect on release of exchangeable Al by decreasing the solution activity of Al. Because more than one mechanism was responsible for Al release in this soil, one of which was relatively independent of the $\log K_{Al}$ of the organic acid, there was a poorer relationship between $\log K_{Al}$ and Al release.

The inconsistent relationship between release of organic P and the $\log K_{Al}$ value of the organic acids was extremely interesting (fig. 4-3). Although oxalate and malate had a large impact on release of organic P, other organic acids with similar or larger $\log K_{Al}$ values had no effect. This suggests that the mechanisms of retention for inorganic and organic P in this soil are different. While

inorganic P seems to be released by a ligand-exchange reaction, another mechanism may be responsible for the release of organic P.

Soluble organic P may make a substantial contribution to the total plant availability in this soil. This is indicated by the fact that it contributed a minimum of 25 % and a maximum of over 85 % of the total P released. The conversion of soluble organic P to an inorganic form available for use by plants requires phosphatase enzymes. However, phosphatases have been found to be almost ubiquitous in soils (Cosgrove 1967; Speir and Ross 1978). Therefore, soluble organic P should probably be included in estimates of P release in these and other soils since our data indicates that overlooking this form of P, excludes a significant and, in some cases, dominant form of P. Future work is needed to identify the form and relative availability of these soluble organic P compounds.

Summary

The release of inorganic P from spodic horizon material dominated by Al was increased by the presence of organic acids that form stable complexes with Al. In general, release of inorganic P increased exponentially with increasing $\log K_{Al}$ values of the organic acid. A threshold value of approximately 4.1 was required before substantial amounts of inorganic P were released. Soluble organic P

contributed between 25 and 85 percent of the total P released from this soil. The amount of soluble organic P released was not related to the $\log K_{Al}$ values of the organic acids. The smaller release of organic P by organic acids with similar or larger $\log K_{Al}$ values suggests that it may not be retained in the same manner as inorganic P. Al release also increased exponentially as the $\log K_{Al}$ of the organic acid increased, although the relationship was more variable than for inorganic P. The increased variability was attributed to release of exchangeable Al that was relatively unaffected by the presence of organic acids. Salicylic acid was a notable exception to the observed trend in release of both Al and P release; despite a large $\log K_{Al}$ value little inorganic P or Al was desorbed.

CHAPTER 5
INFLUENCE OF OXALATE LOADING RATE ON PHOSPHORUS AND
ALUMINUM RELEASE FROM SPODIC HORIZON MATERIAL

Introduction

Oxalic acid is one of the most abundant low-molecular-weight organic acids found in forest soils (Stevenson 1967; Graustein et al. 1977). In a survey of LMW organic acids in forest soils supporting slash and longleaf pine in the Florida flatwoods (Chapter 2), oxalate was abundant in every soil sampled. Oxalate has also been identified as a major component of the suite of organic acids in forest soils supporting Douglas-fir (Pseudotsuga menziesii), Ponderosa pine (Pinus ponderosa), and incense cedar (Libocedrus decurrens) in the western United States (Pohlman and McColl 1988; Cromack et al. 1979); Eucalyptus (Eucalyptus marginata) and radiata pine (Pinus radiata) in Australia (Malajczuk and Cromack 1982); yellow birch (Betula alleghaniensis), beech (Fagus grandifolia) and sugar maple (Acer saccharum) in New Hampshire (Smith 1976); spruce (Picea sp.) and birch (Betula sp.) in Russia (Kaurichev et al. 1963); and Scots pine (Pinus sylvestris) in France (Bruckert 1970a, 1970b).

Oxalate forms stable complexes with both Al and Fe (Martell and Smith 1977). Complexation reactions in solution and at oxide surfaces alter metal speciation (Martell et al. 1988), which may affect biogeochemical cycling of elements, mineral weathering and soil genesis (Huang and Schnitzer 1986; McKeague et al. 1986; Tan 1986). Because of its ability to form stable complexes with Al and Fe, oxalate can also release P from Al- and Fe-hydroxide surfaces through a ligand-exchange mechanism (Goldberg and Sposito 1985). This mechanism was invoked to explain P desorption from spodic and argillic horizons in Chapter 3. The release of P from soils generally increases as the concentration of chelating organic acids in solution increases (Swenson et al. 1949; Nagarajah et al. 1970; Alexander and Robertson 1972; Traina et al. 1986b). This trend was clearly demonstrated for oxalate in Chapter 3. The effect of oxalate on P availability, appears to be important only in the spodic and argillic horizons of flatwoods Spodosols. Phosphorus uptake from these horizons may make a valuable contribution to the nutrition of trees growing on low fertility sites, such as the P deficient soils of the southeastern Coastal Plain.

Oxalate in forest soils originates as exudates from roots, fungi and soil bacteria (Stevenson 1967; Smith 1969; Hodgkinson 1977; Graustein et al. 1977). Leaching from plant litter and decomposing soil organic matter also contributes to the soil solution oxalate content (Stevenson 1982;

Pohlman and McColl 1988). Because oxalate tends to originate at these "point sources", concentrations are generally higher at locations in close proximity to the production source, such as the rhizosphere (Cromack et al. 1979). The oxalate concentration also varies with changes in production and degradation of oxalate, changes in soil water content, and with proximity to the production source. Yet, with current analytical techniques, it is difficult to measure oxalate concentration differences at this spatial scale. Therefore, measurements of oxalate are generally based on bulk extractions or displaced soil solution. Consequently, the actual loading rate on any soil surface is largely unknown. At best, a possible range of loading rates may be calculated from current data. Therefore, a reasonable approach to determining the effect of oxalate on P availability to forest ecosystems is to investigate a plausible range of oxalate loading rates and determine the limits of P release.

The objectives of this chapter were to: 1) investigate the effect of oxalate loading rate (mmol oxalate/kg soil) on P release from spodic horizon soil material; and 2) contrast the pattern of release of Al, inorganic P and organic P at the various oxalate loading rates.

Materials and Methods

Soil Material

Soil material from the Bh horizon of a Pomona series (sandy, siliceous, hyperthermic Ultic Haplaquod) was collected from a single soil pit located in Alachua County, Florida. The same soil was used in the experiments in Chapters 3 and 4. The soil material was air-dried, passed through a 2-mm sieve and stored in a plastic barrel prior to use. Selected chemical and physical properties, determined using standard methods (Page et al. 1982; Klute 1986) are presented in Table 3-1.

Oxalate Loading

The influence of oxalate loading rates from 0.5 to 50 mmol oxalate/kg soil on P and Al release were examined in a series of batch studies. The various oxalate loading rates were obtained by increasing the oxalate concentration of the extracting solution from 0.1 mM to 10 mM. Oxalate solutions of the required concentrations were made from reagent grade Na-oxalate and adjusted to pH 4.3 with HCl. In order to minimize the variability in Al release caused by exchangeable Al that was observed in Chapter 3, the ionic strength of the solutions was standardized by adjusting the conductivity of each solution to 1.8 dS/m with NaCl. This corresponds to an ionic strength of approximately 0.023 M (Lindsay 1979). To examine the effect of a change in solution/soil ratio, several alternate combinations of

solution/soil ratio and oxalate concentration were used to replicate the oxalate loading rates of 5, 10 and 25 mmol/kg.

Triplicate samples of the appropriate amount of air-dried soil were placed in polyethylene bottles and 50 mL of oxalate solution were added to obtain the desired solution/soil ratio and oxalate loading rate. Two drops of toluene were added to retard microbial growth and the bottles were placed on a reciprocating shaker at 100 cycles/minute. After 6 hours, the samples were removed and the solution was filtered through 0.45 micron nylon membrane filters.

Phosphorus Adsorption

The ability of these soils to sorb P was also examined. Duplicate, 10-g samples of soil were equilibrated for 16 hours with 50 mL of 0.01 M CaCl_2 solution containing 0 to 6.46 mM P. The P concentration in the resultant solution was used to calculate the P adsorbed by the soil.

Chemical Analysis

The conductivity of the filtrate was measured with a platinum conductivity electrode on a YSI conductivity meter. Solution pH was measured with a combination glass electrode on an Orion pH meter. Aluminum was determined using flame emission spectrophotometry with a $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame.

Inorganic P in the filtrate was determined by a molybdenum-blue colorimetric procedure using ascorbic acid as a reductant (Murphy and Riley 1962). This is an

operational definition of inorganic P since the molybdenum hydrolyzes some organic P compounds (Stainton 1980; Tarapchak et al. 1982). Total P was also measured in each sample as follows: A 20-mL aliquot of the filtrate was evaporated to dryness at 100 °C in a 50-mL pyrex beaker. The beaker was then placed in a muffle furnace overnight at 500 °C. The beaker was allowed to cool and then placed on a hot plate. A 10-mL aliquot of 40% HCl was added to each sample and evaporated to dryness followed by a 5-mL aliquot of concentrated HCl which was also evaporated to dryness. The beakers were allowed to cool and the sample was redissolved in 20 mL of 0.1 N HCl. Phosphorus in solution was again measured by the Murphy-Riley procedure. Soluble organic P was operationally defined as the difference between Murphy-Riley P in the dry-ashed and acid-digested sample (total P) and Murphy-Riley P in the initial filtrate (inorganic P).

Oxalate Analysis

Oxalate in the filtered solutions was determined by isocratic high pressure liquid chromatography (HPLC) (Lee and Lord 1986). The HPLC system consisted of a Gilson single piston high pressure pump and pressure module (Gilson Medical Electronics, Inc., Middleton, Wisconsin), a Rheodyne model 7125 injection valve fitted with a 20 µL injection loop (Rheodyne, Inc., Cotati, California), a Hamilton PRP-X300 150 x 4.1 mm organic acid column (Hamilton Co., Reno, Nevada), a Gilson Holochrom variable wavelength uv detector,

and a Gilson computerized integrator. Oxalate was eluted at ambient temperature with 0.001 M H_2SO_4 as the eluent at a flow rate of 2 mL/min and detected at 210 nm. Oxalate was quantified by comparing peak areas with an external standard curve.

Results

Release of inorganic P from the Bh horizon soil material increased as the oxalate loading rate increased (fig. 5-1). At low oxalate loading rates, organic P concentrations in the extracts exceeded inorganic P. Desorption of organic P increased more slowly, however, than inorganic P as the oxalate loading rate increased. Therefore, the proportion of organic P in the pool of P released decreased as the oxalate loading increased (fig. 5-2). At low oxalate loading rates, organic P accounted for over 80 % of the P released but dropped to 20 % at high oxalate loading rates. The results of organic P release from this study were quite interesting since soluble organic P was also the dominant form of P released at low oxalate and citrate loading in an Aquic Hapludult supporting radiata pine in New Zealand, yet decreased in proportion as loading rates increased. (Comerford and Skinner 1989).

The influence of the solution/soil ratio on inorganic P release at three oxalate loading rates is presented in fig. 5-3. At each loading rate, desorption of inorganic P increased as the solution/soil ratio increased. At the low

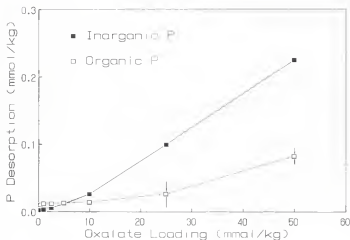


Figure 5-1. Influence of oxalate loading rate on inorganic and organic P desorption from Pomona Bh horizon material. Vertical bars represent ± 1 standard deviation. Where no vertical bars are indicated, the symbol size exceeds the standard deviation.

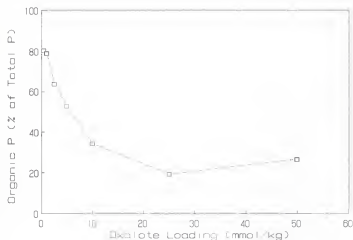


Figure 5-2. Organic P as a percentage of the total P released at the various oxalate loading rates from Pomona Bh horizon material.

oxalate loading rate, the effect of increasing solution/soil ratio was small when the solution/soil ratio was less than 10:1. Changes in solution/soil ratio had almost no effect on organic P release in this soil.

The amount of Al released from the Bh horizon was an order of magnitude greater than the amount of P released. Aluminum release also increased as the oxalate loading rate increased (fig. 5-4). At a given oxalate loading rate, Al release was greater in the sample with the larger solution/soil ratio, although the differences were small (data not presented).

The changes in release of inorganic P and Al associated with increasing solution/soil ratio appeared to be an effect of dilution. At a given oxalate loading rate, the activity of P and Al, in solution would be lower in the samples with higher solution/soil ratios and thus the release of inorganic P and Al would tend to increase. This was a simple example of Le Chatelier's principle.

Oxalate adsorption increased in an approximately linear fashion as oxalate loading increased (fig. 5-5a). Phosphorus adsorption was much greater than oxalate at a given loading rate indicating that this soil has a higher affinity for P than oxalate. The adsorption isotherm for P followed the typical curvilinear pattern while the adsorption isotherm for oxalate was linear (fig 5-5b).

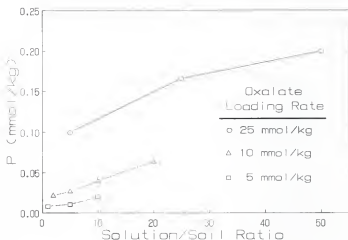


Figure 5-3. Influence of solution/soil ratio on inorganic P desorption from Pomona Bh horizon material at 3 oxalate loading rates. Symbol size exceeds the standard deviation in all cases.

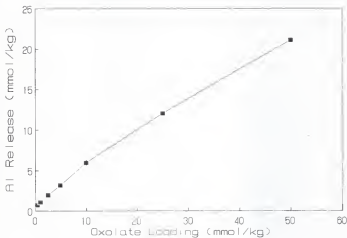


Figure 5-4. Influence of oxalate loading on release of Al from Pomona Bh horizon material. Vertical bars represent ± 1 standard deviation. Where no vertical bars are indicated, the symbol size exceeds the standard deviation.

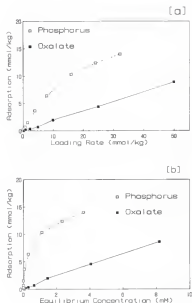


Figure 5-5. Adsorption of oxalate and inorganic P by Pomona Bh horizon material. [a]: Influence of anion loading rate; [b]: Standard adsorption isotherms for inorganic P and oxalate.

Discussion

Oxalate is specifically adsorbed as an inner-sphere complex at Al hydroxide surfaces, and was therefore able to replace inorganic P through a ligand-exchange reaction. This occurred in spite of the greater affinity of the surface for P compared to oxalate demonstrated in figure 5-5. Even at oxalate loading rates as low as 5 mmol/kg, inorganic P release was an order of magnitude greater in oxalate than in water (See Chapter 4). As the oxalate loading rate increases, additional inorganic P apparently becomes susceptible to ligand exchange, perhaps simply as a result of mass action. Ligand exchange at Al-OH surfaces also stimulates Al dissolution (Stumm 1986), and in combination with complexation reactions that lower the activity of Al in solution, probably contributed to the increased release of Al as oxalate loading increased.

The amount of oxalate adsorbed in this experiment was considerably less than the amount sorbed at the same loading rate after a similar reaction period (6 hours) in the kinetics study presented in Chapter 3. The ionic strength of the oxalate solution in this study was considerably higher, however, than in the kinetics study. Barrow (1987 p. 76) showed that the adsorption of P at oxide surfaces decreases as the ionic strength of the background electrolyte increases at pH values below the zero point of charge. This same phenomenon was observed for the Pomona Bh horizon

material when comparing P sorption in 0.01 and 0.001 M CaCl_2 solutions. It seems probable that similar results would occur with oxalate adsorption, perhaps to a greater degree since the surface seems to have a lower affinity for oxalate than P.

Previous work with soils from the southeastern Coastal Plain has shown that spodic horizons contain a significant portion of the soils nutrient capital. Van Rees and Comerford (1986) have documented the proliferation of slash pine roots in the spodic horizon. Using mechanistic nutrient-uptake models, it has recently been shown that a large proportion of the P uptake by a young forest ecosystem could come from the spodic horizon if the sorbed P was in a plant-labile form (Neary et al. In press).

The results in this chapter, which are supported by the data in Chapters 3 and 4, clearly show that P release from spodic horizon material increases in the presence of oxalate and was magnified as the oxalate loading rate increased. This suggests that oxalate does in fact increase the pool of plant labile P in the spodic horizon. The results presented in Chapter 2 have already demonstrated that the oxalate concentrations in the spodic horizon were greater than in the A horizon soil. In combination, these two results highlight the potential importance of subsoil fertility to the productivity of pine plantations in the flatwoods. Based on the work of Comerford et al. (1984) and of Neary et al.

(In press), subsoil horizons may make a large contribution to the amount of P taken up by pine plantations in the flatwoods.

The importance of oxalate in terms of the supply of P to trees must be viewed in the context of the natural rates of oxalate loading encountered in these soils. The oxalate concentrations in the bulk extracts from spodic horizon soil material ranged from 2.0 to 2.8 mM (Chapter 2). Assuming a gravimetric water content of 30 % these concentrations correspond to oxalate loading rates of 0.6 to 0.8 mmol oxalate/kg in the bulk soil. Even at such low loading rates, the release of inorganic P would be significantly increased. Organic P would also comprise a major fraction of the pool of available P at these loading rates.

As previously mentioned, however, bulk extractions tend to dilute the local concentration of oxalate that may exist at microsites in soils. This may be especially true in the vicinity of root and fungal hyphae surfaces that produce large quantities of oxalate. It therefore seems reasonable that at localized sites in close proximity to oxalate sources, the actual loading rate could be one or even two orders of magnitude greater than that calculated from bulk solution concentrations. The oxalate loading rates used in this study may therefore exist under field conditions. Under these circumstances, the supply of P from the spodic horizon would be greatly enhanced.

Conclusions

In light of the above discussion, two conclusions may be drawn from the data in this study. First, the results from this and the preceding chapters conclusively demonstrate that oxalate increases the release of P in spodic and argillic horizons of Spodosols. Even at the low loading rates, there is a substantial increase in inorganic P release. At microsites such as the surface of roots and mycorrhizal fungi, oxalate loading may be orders of magnitude greater with a concomitant increase in release of inorganic P. The implications for increased P availability in the rhizosphere and improved tree nutrition in these soils are clear. Second, soluble organic P is a significant component of the pool of available P in this soil. At the oxalate loading rates normally encountered in bulk soil, organic P may account for between 50 and 90 % of the total P released. The importance to tree nutrition of the soluble organic P depends on its form and on the presence of appropriate phosphatase enzymes. However, phosphatase enzymes are common in soils (Speir and Ross 1978). Organic matter in association with soil surfaces is often stabilized against degradation (Stevenson 1982). The release of organic P into the bulk soil solution by oxalate may be the first step in the mineralization process. Therefore, soluble organic P may need to be included in the estimates of

available P in Spodosols of the southeastern Coastal Plain. (Also see Appendix A).

The data in this chapter were obtained following a single addition of oxalate to the soil. It was shown in Chapter 3 that the effects of oxalate are transitory, particularly in the spodic and argillic horizons. Organic acids are likely to be produced more-or-less continuously in soils. Therefore, information is needed on the effects of repeated oxalate additions on P release in these soils. This question is addressed in the following chapter.

CHAPTER 6
THE EFFECT OF SEQUENTIAL OXALATE ADDITIONS ON
PHOSPHORUS AND ALUMINUM RELEASE FROM TWO SPodosOLS
OF THE SOUTHEASTERN COASTAL PLAIN

Introduction

In Chapter 5, it was shown that the release of P and Al from spodic horizon material increased as the oxalate loading rate increased. At the loading rates studied, oxalate had a greater impact on desorption of inorganic P than organic P. The release of P and Al, however, was measured following a single oxalate addition. The kinetic studies in Chapter 3 indicated that the increased release of P in the presence of oxalate was transitory in these soils. Solution P levels declined rapidly as the oxalate in solution was either sorbed or degraded.

In spite of rapid degradation in soils (Hodgkinson 1977), relatively large steady state concentrations of oxalate are present in southeastern Spodosols (Chapter 2). This suggests that large amounts of oxalate are produced more-or-less continuously in these soils. Repeated additions of oxalate may be important because soil-solution P must be frequently replenished (on an hourly to daily basis) to

meet the continuous P demand of plants (Barber 1984). Therefore, it would be useful to examine the effects of repeated oxalate additions on P release in these soils.

The objectives of this study were to examine the cumulative effect of repeated oxalate additions on P and Al release from surface and subsurface horizons from selected Spodosols of the flatwoods.

Materials and Methods

Soil Material

Soil material from two Spodosols supporting slash pine plantations were used in this study. The soils were a Pomona series (sandy, siliceous, hyperthermic Ultic Haplaquod) located in Alachua County, Florida, and a Leon series (sandy, siliceous, thermic Aeric Haplaquod) from Charlton County, Georgia. Soil samples were collected from individual pedogenic horizons (Leon A and Bh; Pomona A, Bh, and Bt) from a soil pit dug at a single location in each series. The soils were air-dried, passed through a 2-mm sieve, and stored in plastic barrels prior to the experiment. Selected physical and chemical characteristics of these soils determined by standard methods (Page, et al. 1982; Klute 1986) are presented in Table 6-1.

Sequential Desorption Isotherms

The influence of repeated oxalate additions on the release of P and Al was examined using a sequential batch

procedure. Oxalate solutions of 0.0, 0.5 and 5.0 mmol/L were made from reagent-grade Na-oxalate and adjusted to pH 4.3 with HCl. A 5:1 solution/soil ratio was used which resulted in sequential loading rates of 0, 2.5 and 25 mmol oxalate/kg soil, respectively, in each extract. Cumulative oxalate loading rates were determined by multiplying the loading rate by the number of sequential extractions performed.

Duplicate, 20-g samples of air-dried soil material were placed in 250-mL polycarbonate, centrifuge bottles and the weight of the bottle plus soil was recorded. One hundred milliliters of oxalate solution were added along with two drops of toluene and the weight of the bottle was again recorded. The samples were then placed on a reciprocating shaker at 100 cycles per minute for 12 hours. The soil suspension was then allowed to settle for 30 minutes and the supernatant was decanted off and filtered through 0.45 micron nylon membrane filters. Any soil on the filter paper was placed back in the centrifuge bottle and the weight was recorded again to account for entrained extracting solution. One hundred milliliters of oxalate solution were again added to each bottle along with two drops of toluene and the extraction procedure was repeated. A total of five extractions were conducted.

Table 6-1. Selected physical and chemical characteristics of soils used in this study.

Property	Leon Series		Pomona Series		
	A	Bh	A	Bh	Bt
Sand (%)	94	83	93	89	78
Clay (%)	1	6	1	4	18
Organic C (%)	0.98	0.90	1.77	2.19	0.31
pH (H ₂ O)	4.22	4.38	4.03	4.23	4.74
<u>P (mg/kg)</u>					
Total	18.0	40.7	21.8	65.5	138.6
Total Organic	8.5	34.1	9.6	49.2	27.1
Mehlich 1	3.5	7.9	5.1	7.6	9.7
Water	5.4	0.5	9.1	0.4	0.1
0.01 M CaCl ₂	4.0	1.0	7.0	2.0	1.0
<u>Potassium (mg/kg)</u>					
Mehlich 1	4	0.1	13	3	2
<u>Calcium (mg/kg)</u>					
Mehlich 1	34	12	111	13	14
<u>Magnesium (mg/kg)</u>					
Mehlich 1	12	1	30	4	8
<u>Al (mg/kg)</u>					
Acid Oxalate	113	961	226	1357	1075
Pyrophosphate	90	904	215	1379	769
Mehlich 1	31	153	37	432	230
1 N KCl	7	107	15	158	118
Water	4	8	2	13	3
<u>Fe (mg/kg)</u>					
Acid Oxalate	15	6	12	6	9
Pyrophosphate	32	17	35	19	89
Mehlich 1	2	2	3	2	22
Water	0.6	0.9	0.7	0.6	0.8

Phosphorus and Aluminum Analysis

Inorganic P in the filtrate was determined by a molybdenum-blue colorimetric procedure using ascorbic acid as a reductant (Murphy and Riley 1962). This is an operational definition of inorganic P since the molybdenum hydrolyzes some organic P compounds (Stainton 1980; Tarapchak et al. 1982). Total P was also measured in each sample as follows: A 20-mL aliquot of the filtrate was evaporated to dryness at 100 °C in a 50-mL pyrex beaker. The beaker was then placed in a muffle furnace overnight at 500 °C. The beaker was allowed to cool and then placed on a hot plate. A 10-mL aliquot of 40% HCl was added to each sample and evaporated to dryness followed by a 5-mL aliquot of concentrated HCl which was also evaporated to dryness. The beakers were allowed to cool and the sample was redissolved in 20 mL of 0.1 N HCl. Phosphorus in solution was again measured by the Murphy-Riley procedure. Soluble organic P was operationally defined as the difference between Murphy-Riley P in the dry-ashed and acid-digested sample (total P) and Murphy-Riley P in the initial filtrate (inorganic P).

Aluminum in the filtrates was determined using flame emission spectrophotometry. A $\text{N}_2\text{O} - \text{C}_2\text{H}_2$ flame was employed to reduce interferences (Barnhisel and Bertsch 1982).

Results

Sequential P Release

The sequential desorption of P from the Pomona and Leon soils in the three oxalate solutions is presented in fig. 6-1 and 6-2. Sequential desorption of P indicates the ability of a soil to replenish P removed from solution and is a measure of the P buffer power of the soil. The general pattern of both inorganic and organic P desorption in soil from the same pedogenic horizon in the two soils was similar. As the oxalate concentration increased, greater amounts of P were released. In the A horizons, very little P was released after the initial extraction and the effect of oxalate was small. This suggests that the P buffer power of the A horizons was also small. In the Bh and Bt horizons, release of inorganic P was negligible in water. The amount of organic P released in water was greater than the amount of inorganic P in the Bh horizon. In contrast, in the oxalate solutions, P desorption increased in each sequential extract and greater amounts of P were released in the 5.0 mM oxalate solution than in the 0.5 mM oxalate solution. In the Pomona Bt horizon, the effect of oxalate on release of inorganic P was similar to that for the Bh horizon; however, release of organic P was negligible. In the Bh and Bt horizons, there was apparently very little

Figure 6-1. The effect of oxalate on the sequential desorption of P from a Pomona series soil at a solution / soil ratio of 5:1. Vertical bars represent ± 1 standard deviation. Where no vertical bars are indicated, the symbol size exceeds the standard error.

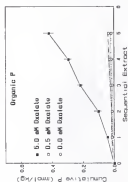
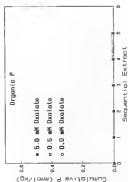
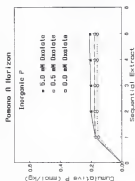
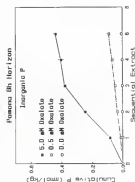
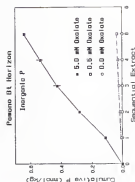
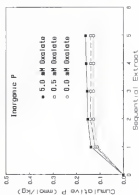
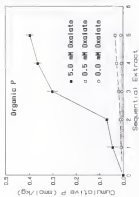
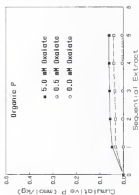
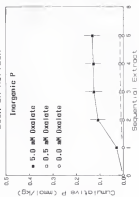


Figure 6-2. The effect of oxalate on the sequential desorption of P from a Leon series soil at a solution / soil ratio of 5:1. Vertical bars represent ± 1 standard deviation. Where no vertical bars are indicated, the symbol size exceeds the standard error.

Lean R Horizon



Lean Bb Horizon



P buffer power in water. When oxalate was added, the P buffer power of the subsoils increased substantially.

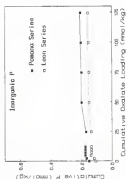
Cumulative Oxalate Loading

In the present study, the cumulative oxalate loading on the soil increased with each sequential extract. The cumulative oxalate loading in the 0.5 mM oxalate solution ranged from 2.5 to 12.5 mmol/kg, while the cumulative loading in the 5.0 mM oxalate solution ranged from 25 to 125 mmol/kg. If P release is controlled by the oxalate loading rate, the sequential release of P at the two solution concentrations in this study should constitute two portions of the same release curve.

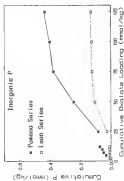
Release of inorganic and organic P in the sequential extracts is plotted against cumulative oxalate loading in fig. 6-3. It appears that there are individual release curves for both inorganic and organic P in each soil which depend on the overall oxalate loading rate. Due to the oxalate concentrations used, there was a gap in the oxalate loading rate between 12.5 and 25 mmol/kg. The P-release data from the one-time oxalate loading study in Chapter 5, which included oxalate loading rates in the missing range, are plotted along with the data from the Pomona Bh horizon in figure 6-4. The combined data from both experiments follow the same P-release curve, supporting the conclusion that

Figure 6-3. The effect of cumulative oxalate loading rate on the desorption of P from Leon and Pomona series soils.

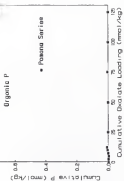
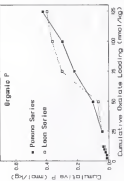
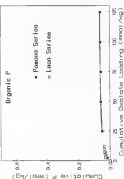
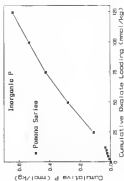
A Horizon Soil



Bh Horizon Soil



BL Horizon Soil



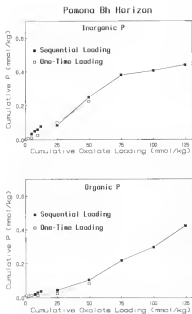


Figure 6-4. Effect of oxalate loading rate obtained by sequential and "one-time" additions on [a] inorganic P desorption and [b] organic P desorption from a Pomona Bh horizon soil.

inorganic and organic P release is controlled by the total oxalate loading rate. The method of loading, one-time or cumulative has relatively little effect on either release of inorganic or organic P.

The effects of oxalate loading on inorganic and organic P desorption in the two soils are summarized below.

Inorganic P (fig. 6-3)

The cumulative amount of inorganic P desorbed in both the A and Bh horizons was greater in the Pomona soil than the Leon soil at all oxalate loading rates. In the A horizon, increasing the oxalate loading rate had little effect on release of inorganic P. In contrast, desorption of inorganic P increased as the oxalate loading rate increased in the Bh and Bt horizons. In the Leon Bh horizon, inorganic P release increased gradually and reached a plateau at an oxalate loading rate of 50 mmol/kg. In the Pomona Bh horizon, release of inorganic P increased more rapidly as the oxalate loading rate increased. Although a true plateau was not reached at the loading rates used in the Pomona Bh, smaller amounts of P were released at loading rates above 75 mmol/kg. Release of inorganic P in the Pomona Bt horizon followed a similar pattern as in the Pomona Bh horizon at oxalate loading rates up to 75 mmol/kg. Unlike the Bh horizon, however, inorganic P release did not fall off at higher loading rates. It was interesting that at high oxalate loading rates, release of inorganic P in the Bh and

Bt horizons approached, and in the Pomona series even exceeded, the amount of inorganic P released in the A horizons.

Organic P (fig. 6-3)

The desorption of organic P in the A horizons was almost identical in the Leon and the Pomona series. As with inorganic P, increasing the oxalate loading rate had little effect on release of organic P in the A horizon. Organic P accounted for between 20 and 30 % of the total P released in the A horizon. In both soils, release of organic P in the Bh horizon increased as the oxalate loading rate increased; however, the pattern of organic P release was different. In the Pomona Bh horizon, organic P release increased almost linearly as the oxalate loading rate increased. In the Leon series there was a large jump in organic P release between 50 and 75 mmol oxalate/kg loading, which caused the release curve to assume a sigmoidal shape. The relative contribution of organic P to the total pool of P released in the Bh horizon depended on the oxalate loading rate. At low loading rates, organic P comprised over 75 % of the P released P in the Bh horizon. This dropped to approximately 30 % at the 50 mmol/kg oxalate loading rate, and then increased once more to between 50 and 75 % of the total pool of P released at the high oxalate loading rates. In the Pomona Bt horizon, desorption of organic P was negligible and was not affected by oxalate loading.

Aluminum

Aluminum also followed a single release curve in each soil that depended only on the total oxalate loading rate (fig. 6-5). As with P, this conclusion was supported by the data on oxalate loading and Al release in the Pomona Bh horizon from Chapter 4 which covered the missing loading rates in the present study (fig. 6-5). In all soils, the release of Al increased as the oxalate loading rate increased, although the amount of Al released from the A horizons was much less than from the Bh and Bt horizons. Aluminum release was also consistently greater in the Pomona series than the Leon series in both the A and Bh horizons.

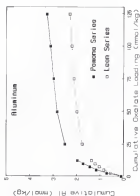
Discussion

Aluminum

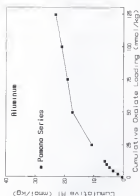
The influence of cumulative oxalate loading on aluminum release in these Spodosols has important implications to the genesis of Spodosols. Additions of organic acid from sources such as the forest floor and plant roots generally occur continuously throughout the year; however, the concentration in soil solution at any one time may be quite small. The cumulative effect of oxalate indicates how, over a pedogenic time frame, large amounts of Al can be stripped from surface soil horizons and translocated through the profile by relatively small, incremental, organic acid additions.

Figure 6-5. The effect of cumulative oxalate loading rate on the release of Al from A horizon, Bh horizon, and Bt horizon soils from Leon and Pomona series. The data for the Pomona Bh horizon includes loading rates obtained from sequential and one-time oxalate additions.

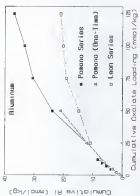
A Horizon Soil



Bt Horizon Soil



Bh Horizon Soil



Phosphorus Desorption

The fact that, even at low loading rates, oxalate increases P release from spodic horizon soil material was clearly demonstrated in the preceding chapters. As the oxalate loading rate increased, P release increased. These results were based, however, on a single oxalate addition. The results in this present chapter extend the observations on the effects of oxalate to repeated additions. This is important because the effects of oxalate on P release were ephemeral (Chapter 3) and oxalate is produced more-or-less continuously in soils (Smith 1969, Stevenson 1967).

In the Bh and Bt horizon, the increase in P buffer power in the presence of oxalate would substantially increase the uptake potential for P. The effect of buffer power is probably more important to the nutrition of perennial plants such as trees which must continuously take up P over decades from the same soil. The effect of oxalate was similar between inorganic P and organic P in the Bh horizon which also suggest that organic P may make a valuable contribution to the supply of available P. The increased buffer power in the Bh and Bt horizons in the presence of oxalate provides support to the hypothesis that subsurface horizons make a large contribution to the P nutrition of trees (Comerford et al. 1984). The low buffer power in the A horizon suggests that, in the short term, available P could be depleted by actively growing trees and

associated understory that have a large P uptake requirement. The pool of available P is also subject to leaching and may be lost from the system. This was observed by Humphreys and Pritchett (1971) following the addition of P fertilizers to similar soils.

Another important result from this study was that the effects on P release of repeated additions of oxalate were cumulative. This has important implications for long term P availability to trees in the flatwoods. Repeated additions of oxalate, even at low individual loading rates, substantially increases the cumulative amount of P available in the soil. On an annual basis, the cumulative amount of oxalate released into the soil may actually be quite large. Two examples, illustrate the types of oxalate loading rates that may occur in soil supporting pine trees:

In the first, the data on oxalate release by Pinus radiata seedlings reported by Smith (1969) was used to calculate the cumulative loading rate. An average seedling produced 200 μg of oxalate over a 10-day period. Assuming a 0.5-mm thick zone of influence around a 2-mm diameter root segment 50-mm long, the oxalate loading rate in a soil with a bulk density of 1.4 g/cc was calculated to be 0.08 $\text{mmol}\cdot\text{kg}^{-1}$ over a period of 10 days. Continuous production of oxalate at this rate would result in a cumulative oxalate loading rate of approximately 30 $\text{mmol}\cdot\text{kg}^{-1}\cdot\text{yr}^{-1}$.

In the second example, the oxalate concentration reported in soil solution from the rhizosphere of slash pine in Chapter 3 was used to calculate the oxalate loading rate. An oxalate concentration of 2.5 mM at a gravimetric moisture content of 30 % results in a steady state loading rate of approximately $0.75 \text{ mmol} \cdot \text{kg}^{-1}$. Assuming that the entire pool of oxalate is replenished on a weekly basis, which is perhaps conservative based on the data in Chapter 3, the cumulative annual oxalate loading rate would approach $40 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{yr}^{-1}$.

The use of annual loading rates is appropriate in pine ecosystems of the flatwoods because P uptake continues throughout the year (Comerford et al. 1984). At an annual, cumulative loading rates of 30 - 40 $\text{mmol} \cdot \text{kg}^{-1}$, the amounts of P released in the Bh and Bt horizons would be similar to that released in the A horizon soil. At the higher loading rates, the relative contribution of organic P also becomes much more important in the spodic horizons, particularly in the Leon series. This suggests that the influence of organic acid loading on the amount and forms of P released may have a large impact on tree growth in the different soils. Based on the data in figure 6-3, the Pomona soil would probably be a more productive soil than the Leon soil if P were limiting tree growth.

As discussed in Chapter 5, the loading rates calculated above are probably conservative estimates of the actual

oxalate loading rate. Because organic acids tend to originate at localized sources in the soil, such as the root surface, the effective loading rate for soil in the immediate vicinity of these "point sources" may be an order of magnitude or more greater than those calculated above. The importance of subsoils in supplying P to tree roots is magnified under these conditions.

Given these results, it is clear that subsoil horizons can play an important role in the P nutrition of pine plantations in the flatwoods. This supports the work of Comerford et al. (1984) and Neary et al. (In press) who reached this same conclusion based on the theoretical results of a mechanistic model that estimated P uptake by slash pine roots. In combination with the greater moisture availability in the subsoil (Phillips et al. In press), the increased P availability and P buffer power due to oxalate highlights the importance of the subsoil in determining the productivity of pine plantations in the flatwoods of the southeastern United States.

The results from this chapter also highlight an interesting facet of P chemistry in the Spodosols of the flatwoods. Although the pool of available P is large in the A horizon soil, these soils possess almost no buffer capacity. Their entire pool of inorganic P is essentially in a water soluble form. The data of Burger (1979) and Morris (1981), however, indicate that leaching and plant uptake do

not seem to deplete inorganic P in these soils. This leads one to speculate as to the source of inorganic P in the A horizons. Mineralization of organic P seems a likely source of inorganic P and may be the mechanism buffering soil solution P in the A horizons. This is supported by the high temperature and moisture regime in the flatwoods that is conducive to rapid mineralization rates (Burger 1979). In this regard, it is interesting that Gholz et al. (1985) have reported net immobilization of P in the forest floor of slash pine plantations throughout a chronosequence of stands representing an entire rotation. These incongruous results suggest that additional work on the effects of desorption, mineralization, leaching and plant uptake of P are needed in A horizons of Spodosols of the flatwoods.

Conclusions

There are two important results from the sequential P desorption data. The first was the increased buffer capacity in the Bh and Bt horizons when oxalate was added. This applied to both inorganic and organic P in the Bh horizon. Second, the effects of sequential additions of oxalate on P desorption were cumulative. Over the course of a year, the cumulative effect of low oxalate loadings substantially increases the amount of P available for plant uptake from the subsurface horizons. These results again suggest that spodic and argillic horizons play an important role in the P

nutrition of forest ecosystems growing on Spodosols of the southeastern United States.

The effects of oxalate on organic P were as great or greater than the effects on inorganic P in the Bh horizons. The different patterns of organic and inorganic P desorption suggests, however, that oxalate affects organic P in a different way than inorganic P. Additional work is needed to identify the forms of organic P released by oxalate in these soils.

Since the Pomona soil released considerably more P at comparable oxalate loading rates than the Leon soil, it would be interesting to compare P uptake from these two soils. If P uptake from soil could be correlated with a set of parameters describing the effects of oxalate loading on P desorption, it might be possible to use these parameters to rank the productivity of soils in the flatwoods and estimate the need for P fertilizer. A mechanistic nutrient-uptake model such as Barber's (1984) might be a useful in this regard. Based on the effects of oxalate on C_{11} and b, greater P uptake would probably be predicted from the Pomona Bh horizon than the Leon Bh horizon. Confirmation of these predictions, perhaps in a greenhouse study utilizing individual soil horizons, would suggest that tree seedlings are in fact able to modify the soil and thereby increase P availability.

CHAPTER 7 OVERALL CONCLUSIONS

The following is a list of the major results and conclusions from this research by chapter:

Chapter 2

1. Oxalate and formate were the dominant organic acids identified in soils from the flatwoods of the southeastern Coastal Plain.
2. Concentrations of both oxalate and formate were higher in the Bh and Bt horizons than the A horizons in the soils studied.

Chapter 3

1. Inorganic P was not tightly held at Al-oxide surfaces in the A horizon of a Pomona soil. As a result, organic acids had little direct effect on the kinetics of inorganic P release.
2. Inorganic P was tightly held in the Bh and Bt horizons of the Pomona soil. Release of inorganic P from the Bh and Bt horizons was negligible in water and in formate. Oxalate, however, was able to release significant amounts of P by what appeared to be ligand-exchange reactions at Al-oxide surfaces.

3. The effects of oxalate on P desorption were ephemeral. In all three horizons, release of inorganic P reached a peak between 6 and 12 hours and then declined. The drop in inorganic P levels began immediately after all the added oxalate had been sorbed.
4. Calculated ion activity products (IAP) suggest that the drop in P concentrations in all horizons was associated with the formation of amorphous $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$.
5. Oxalate increased the desorption of organic P in both the A and Bh horizons of the Pomona series soil. Organic P was negligible in the Bt horizon.

Chapter 4

1. The desorption of inorganic P from spodic horizon soil material was increased by the presence of organic acids that form stable complexes with Al. Release of inorganic P was an order of magnitude greater in 1.0 mM oxalate than in water.
2. In general, release of inorganic P increased exponentially with increasing $\log K_{\text{Al}}$ value of the organic acids. Among the 16 organic acids studied, a threshold value for $\log K_{\text{Al}}$ of between 4.0 and 4.5 was required before substantial amounts of P were released.
3. Soluble organic P contributed between 25 and 85 % of the total P released in this soil.
4. Only oxalate, malate and formate increased the amount of organic P released from the spodic horizon. The smaller release of organic P by organic acids with similar or larger

$\log K_{Al}$ values suggests that organic P is not retained in the same manner as inorganic P.

5. Al release also increased exponentially as the $\log K_{Al}$ values of the organic acids increased; however, the relationship was more variable than for P. The release of exchangeable Al was believed to cause the greater variability.

Chapter 5

1. Release of both inorganic and organic P from spodic horizon material increased as the oxalate loading rate increased. Through an oxalate loading rate of 50 mmol/kg, desorption of inorganic P increased to a greater extent than organic P.

2. At low oxalate loading rates, < 5 mmol oxalate/kg soil, release of organic P was greater than inorganic P, accounting for over 80 % of the total P desorbed. As a consequence of the differential effect of oxalate, the proportion of total P accounted for by organic P dropped from 80 % at a loading rate of 0.1 mmol/kg to approximately 20 % at a loading rate of 25 mmol/kg.

3. Release of Al also increased as the oxalate loading rate increased. At the same oxalate loading rate, the amount of Al released was two orders of magnitude greater than the amount of P released.

4. The surfaces in the Bh horizon had a much greater affinity for P than for oxalate. At equivalent loading

rates, considerably more P than oxalate was sorbed by this soil.

Chapter 6

1. The effects of repeated oxalate additions on P desorption were cumulative. The same amount of P was desorbed regardless of the method of loading; i.e. one-time loading vs repeated additions. The important feature determining the amount of P released was the oxalate loading rate expressed as mmol/kg soil. Over the course of a growing season, repeated additions of oxalate can significantly increase P availability in subsurface horizons.
2. Oxalate had little effect on release of P from A horizon soil from either soil studied. The A horizon soils had essentially no P buffer capacity.
3. Oxalate substantially increased release of inorganic P in both the Bh and Bt horizons. At the highest oxalate loading rates, release of inorganic P from the Bh and Bt horizons was greater than from the A horizons.
4. The P buffer capacity of the subsoils was negligible in water; however, it was large in the oxalate solutions.
5. Release of organic P in the Bh horizons was substantial. At the high oxalate loading rates, release of organic P in the Pomona Bh horizon was as great as inorganic P while in the Leon Bh horizon release of organic P exceeded that of inorganic P.

Implications for Tree Nutrition

These results have potential implications for tree nutrition and forest productivity in the flatwoods since P often limits tree growth in the flatwoods.

Oxalate increases P release from spodic and argillic horizons. Higher oxalate concentrations were also shown to exist in these subsoil horizons. These two results, in combination with the work of Van Rees and Comerford (1986) showing that roots of slash pine tend to proliferate in the subsoil of flatwoods Spodosols, lend support to the work of Comerford et al. (1984) and Neary et al. (In press) that subsurface horizons may contribute a substantial portion of the P taken up by trees growing in the flatwoods. In this regard, the contribution of organic P to the pool of plant-available P magnifies the importance of the spodic horizon.

Future Research

The results of this research illustrate the potential effect of oxalate on P availability in these soils. The next logical step would be to determine if this mechanism is in fact required to account for the P-uptake patterns of trees. One way to proceed would be to incorporate the effects of oxalate into a mechanistic modeling approach such as that of Barber (1984). This might be done through the effects which oxalate has on initial solution P concentration (C_{11}) and on the P buffer power of the soil (b). If the effect of oxalate

on these parameters needs to be included in the model to accurately predict P uptake, it would lend support to the conclusion that tree roots do in fact alter the soil and increase plant-available P through oxalate exudation.

If the above approaches were successful, it might be possible to use the effects of oxalate on P release as a way of evaluating soil P fertility and the need for P fertilization. Eventually, this approach may lead to an accurate diagnostic technique for predicting P response of trees in the flatwoods. Unfortunately, improved diagnostic techniques for P require a clear understanding of water supply and movement in the soil. Our knowledge in this area lags far behind even our meager understanding of P chemistry in the soils of the flatwoods.

Finally, work needs to be done on the soluble organic P fraction in these soils. Until the forms of organic P are identified, it will not be possible to get beyond a first approximation of the role that organic P plays in P availability and forest productivity in the flatwoods.

APPENDIX
ACID PHOSPHATASE ACTIVITY AND PHOSPHATASE HYDROLYZABLE
PHOSPHORUS IN TWO SPodosOLS

Introduction

A large proportion of the total P in surface soils often exists in an organic form (Anderson 1980). Organic P can also be a major component of the P released in soil extracts, sometimes exceeding the amount of inorganic P released. In a pioneering study by Pierre and Parker (1927), organic P content in the displaced soil solution from 20 soils averaged 0.47 mg/L while inorganic P averaged 0.09 mg/L. Numerous studies have since shown that the amount and proportion of soluble organic P depends on both the properties of the soil and on the extractants used (Martin 1964; Wild and Oke 1966; Martin 1969; Haynes and Swift 1985; Comerford and Skinner 1989).

In the main body of this dissertation, the effects of oxalate on release of both organic and inorganic P from selected Spodosols of the Southeastern Coastal Plain were examined. Organic P was a significant component of the P released from both surface and spodic horizon material. In A horizon material, organic P comprised 20 to 30 % of the P released in water. In the Bh horizon, organic P made up over 80 % of the P released in water. Release of both inorganic

and organic P increased in the Bh horizon in presence of oxalate, however, the pattern of release between the two forms differed. As a consequence, the proportion of organic P decreased from approximately 80 % at in the absence of oxalate to 20 % at 25 mmol oxalate/kg and then increase to over 50 % at 125 mmol oxalate/kg soil.

Organic P is therefore an important component of the pool of soluble P in Spodosols of the southeastern Coastal Plain. Organic P has also been shown to be important in desert, grassland, and forest ecosystems (Cole et al. 1977; Wood et al. 1984; Smeck 1985; Lajtha and Schlesinger 1988). The cycling of organic P has a large impact on P availability and on long-term ecosystem productivity (Halstead and McKercher 1975). Turner and Lambert (1985) suggested that soluble organic P may be the most important form of P affecting long term forest productivity of Pinus radiata in Australia.

Although some organic P can be absorbed directly by plants, normally it must be converted to orthophosphate before it can be utilized (Hayman 1975; Tate 1984). Organic P compounds are mineralized through an enzyme mediated process that hydrolyzes C-O-P ester bonds (Cosgrove 1967). In acid soils, acid phosphatases predominate (Tabatabai 1982). Acid phosphatases are classified into two broad groups, phosphomonoesterases and phosphodiesterases, based on the form of P ester bond hydrolyzed (Speir and Ross

1978). Although certain phosphatases are nonspecific and cleave a variety of P ester bonds, most phosphatases hydrolyze a specific form of organic P.

Plant roots, mycorrhizal fungi and bacteria all produce phosphatases in the soil (Speir and Ross 1977; Dighton 1983; Tarafdar and Claassen 1988). Phosphatase production is stimulated by organic matter and by organic P compounds (Speir and Ross 1978). In contrast, inorganic P is a competitive inhibitor of acid phosphatase in soils (Speir and Ross 1978; Tabatabai 1982). Elevated inorganic P concentrations in soil solution inhibit phosphatase activity in soils. Pang and Kolenko (1978) observed decreased phosphatase activity in soils supporting Douglas-fir stands in British Columbia following P fertilization.

Considerable emphasis has been placed on P availability in the rhizosphere (Curl and Truelove 1986; Marschner et al. 1986). Large quantities of P-containing organic compounds can be introduced into the rhizosphere as root exudates, microbial biomass and root debris (Rovira and McDougall 1967; Rovira and Davey 1974; Curl and Truelove 1986). Increased phosphatase activity in the rhizosphere has been observed for wheat, onion, clover and rape (Hedley et al. 1982; Tarafdar and Jungk 1987). Given the high percentage of organic P in southeastern Spodosols, it is reasonable to argue that phosphatase activity in the rhizosphere of slash

pine roots, if present, could be an important factor increasing P availability.

The objectives of this work were to: 1) examine the changes in acid phosphatase activity in the rhizosphere of slash pine seedlings growing in soil from surface and subsurface horizons of selected Spodosols of the southeastern United States. 2) examine the effect of increased inorganic P on acid phosphatase activity in these soils; and 3) examine the amount of water soluble organic P released in these soils that was hydrolyzed by a specific acid phosphomonoesterase.

Materials and Methods

Soil Material

Soil material from two Spodosols supporting slash pine plantations were used in this study. The soils were a Pomona series (sandy, siliceous, hyperthermic Ultic Haplaquod) located in Alachua County, Florida, and a Leon series (sandy, siliceous, thermic Aeric Haplaquod) from Charlton County, Georgia. Soil samples were collected from individual pedogenic horizons (Leon A and Bh; Pomona A, Bh, and Bt) from a soil pit dug at a single location in each series. These soils have been used in our previous work on the influence of organic acids on P release. The soils were air dried, passed through a 2-mm sieve, and stored in plastic barrels prior to the experiment. Selected physical and

chemical data characterizing these soils are presented in Table 6-1.

Acid Phosphatase Activity in Rhizosphere and Bulk Soil

Triplicate, three-kg samples of air-dried soil material from each horizon were placed in individual 15-cm diameter by 15-cm deep plastic pots. The pots were fertilized with P at three rates (0, 6.67 and 13.33 mg P/kg soil) with reagent-grade potassium phosphate. The soil in the pots was then wetted to field capacity with tap water and allowed to equilibrate for two weeks.

Half-sib seed from genetically improved slash pine (UF 84-57) were obtained from the Cooperative Forest Genetic Research Program at the University of Florida. The seeds were soaked in distilled water for 24 hours and then placed on moist perlite in an enclosed plastic chamber and allowed to germinate. Seedlings were transplanted to the pots after primary needles had emerged but before the seed coats were shed. Six seedlings were planted in each pot and then thinned to four after two weeks. Seedlings were grown for six months (January through June 1988) in a greenhouse in Gainesville, Florida under natural light. Pots were watered as needed with tap water and excess water was allowed to drain by gravity. At monthly intervals, 100 mL of a "minus P" nutrient solution ($0.004 \text{ M Ca(NO}_3)_2 \cdot 4\text{H}_2\text{O}$; $0.002 \text{ M NH}_4(\text{SO}_4)$; 0.006 M KNO_3 ; and $0.001 \text{ M MgSO}_4 \cdot 7\text{H}_2\text{O}$) were added to each pot. Seedlings were sprayed with malathion in

February to control aphids and in May to control spider mites.

After six months growth, the seedlings were lifted from the pots. The entire root system from all four seedlings in each pot was treated as one unit. Rhizosphere soil was separated from bulk soil by gently shaking the root system. Soil adhering to the root system was defined as rhizosphere soil while the non-adhering soil was defined as bulk soil (Rovira and McDougall 1967). Rhizosphere soil was subsequently separated from the roots by a more vigorous shaking. Soil still adhering to the roots was removed by hand with a dissecting probe.

Rhizosphere and non-rhizosphere soil from each pot was placed in separate polyethylene plastic bags and immediately placed on ice. Samples were stored overnight at 3 °C. Analyses were initiated the following day.

The activity of acid phosphomonoesterase in all soils was assayed following the procedure of Tabatabai (1982). Acid phosphatase activity was measured as the amount of p-nitrophenyl phosphate converted to p-nitrophenol after a one-hour incubation at pH 6.5 and 37 °C. All analyses were performed on moist soil samples. Gravimetric moisture content of each soil was determined (Gardner 1986), and used to correct results to a dry-weight basis.

Water-Soluble P Hydrolyzed by Acid Phosphatase

A second experiment was conducted to determine the proportion of water-soluble organic P released in these soils that was hydrolyzable by a selected acid phosphatase. Triplicate, 25-g samples of air-dried soil from A and Bh horizons of the Leon and Pomona soils were placed in individual 125-mL plastic bottles. One hundred milliliters of distilled water adjusted to pH 4.3 with HCl, were added to each bottle and the bottles were shaken on a reciprocal shaker for 6 hours. The water extracts were filtered through 0.45 micron nylon membrane filters and analyzed immediately.

Inorganic P in the filtrate was determined by a molybdenum-blue colorimetric procedure using ascorbic acid as a reductant (Murphy and Riley 1962). This is an operational definition of inorganic P, since the molybdenum hydrolyzes some organic P compounds (Stainton 1980; Tarapchak et al. 1982). Total P was also measured in each sample as follows: A 20-mL aliquot of the filtrate was evaporated to dryness at 100 °C in a 50-mL pyrex beaker. The beaker was then placed in a muffle furnace overnight at 500 °C. The beaker was allowed to cool and then placed on a hot plate. A 10-mL aliquot of 40% HCl was added to each sample and evaporated to dryness followed by a 5-mL aliquot of concentrated HCl which was also evaporated to dryness. The beakers were allowed to cool and the sample was redissolved

in 20 mL of 0.1 N HCl. Phosphorus in solution was again measured by the Murphy-Riley procedure. Soluble organic P was operationally defined as the difference between Murphy-Riley P in the dry-ashed and acid-digested sample and the initial inorganic P in the filtrate.

Soluble organic P hydrolyzed by an acid phosphatase was determined as follows: A solution of acid phosphatase (orthophosphoric monoester phosphohydrolase [acid optimum] EC 3.1.3.2) derived from wheat germ (Sigma #P3627) containing 1 unit of acid phosphatase per mL was made by dissolving 0.1923 g in 100 mL of distilled water. One unit of acid phosphatase hydrolyzes 1.0 micromole of p-nitrophenyl phosphate per minute at pH 4.8 and 37 °C. A 10-mL aliquot of the soil water extract was placed in a 20-mL glass scintillation vial and 0.1 mL of the acid phosphatase solution was added. The scintillation vial was capped and placed in an incubator at 37 °C for 12 hours. Water soluble organic P concentrations were expected to be less than 0.1 micromole/mL. Therefore, the amount of acid phosphatase added should have been adequate to hydrolyze the entire pool of water-soluble organic P during the 12-hour incubation. After the incubation period, the inorganic P in solution was again determined by the Murphy-Riley Procedure. Organic P hydrolyzed by the added phosphatase was detected as an increase in the measured levels of inorganic P.

Statistical Analysis

Analysis of variance (ANOVA) was used to test for differences between rhizosphere and non-rhizosphere soil in the first experiment. The rhizosphere and non-rhizosphere data were analyzed as subplots in a split plot experiment with soil as the main plot in a randomized complete block design with 3 blocks (Gomez and Gomez 1984). LSD values were used to compare main plot and subplot means (Gomez and Gomez 1984).

The results from the study of phosphatase hydrolyzable P were also analyzed using ANOVA. The study was 2 x 2 factorial established in a random complete block design with three replications.

Statistical analyses were performed using the GLM procedure of the Statistical Analysis System (SAS). LSD values were calculated by hand for the split plot experiment. Computing was done using the facilities of the Northeast Regional Data Center of the State University System of Florida, located on the campus of the University of Florida in Gainesville.

Results

Acid Phosphatase Activity

Acid phosphatase activity in the non-rhizosphere soil was similar in all the soils and was not strongly affected by P fertilization (figs. A-1, A-2). In the absence of added

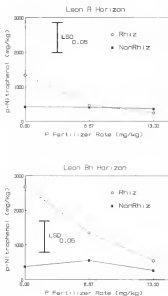
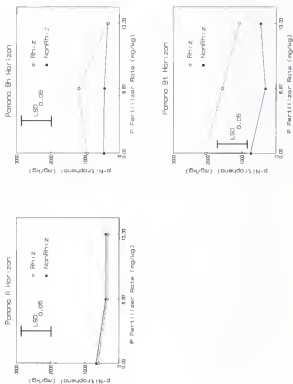


Figure A-1. Acid phosphatase activity in the rhizosphere and nonrhizosphere of slash pine in Leon A and Bh horizon soil as influenced by P fertilization.

Figure A-2. Acid phosphatase activity in the rhizosphere and nonrhizosphere of slash pine in Pomona A, Bh and Bt horizon soil as influenced by P fertilization.



P, acid phosphatase activity in the Leon A and Bh horizon material was greater in the rhizosphere than in the non-rhizosphere (fig. A-1). In the non-P-fertilized soil from the Pomona series, only in the Bt horizon was acid phosphatase activity greater in the rhizosphere (fig. A-2). Acid phosphatase activity in the rhizosphere decreased as the P fertilization rate increased. At the high P rate, there were no longer any differences in acid phosphatase activity between the rhizosphere and non-rhizosphere in any of the soils.

Acid Phosphatase Hydrolysis of Organic P

The amount of water-soluble inorganic P was an order of magnitude greater in the A horizon than the Bh horizon in both the Leon and the Pomona series (Table A-1). The amount of water soluble-organic P, however, was similar in the A and Bh horizons from the two series. Although the absolute amounts were similar, organic P made up a significantly larger proportion of the total water-soluble P in the Bh horizon material. Over 75 % of the P release in the Bh horizons was in an organic form while less than 25 % was in an organic form in the A horizons. Between 20 and 30 % of the water-soluble organic P in these soils was hydrolyzed by the added acid phosphomonoesterase. Although 3 to 4 times more soluble organic P was hydrolyzed in the A horizons compared to the Bh horizon, the difference was not significant.

Table A-1. Water-soluble inorganic P, organic P and acid phosphatase hydrolyzable P from A and Bh horizon soil material from Leon and Pomona soils

Soil	Inorganic P	Organic P		Acid Phosphatase Hydrolyzable Organic P	
		Amount	% of total		
	(mmol/kg)	(mmol/kg)	(%)	(mmol/kg)	(%)
A Horizon Soil					
Leon	0.0898	0.0284	24	0.0084	30
Pomona	0.1432	0.0265	16	0.0060	23
Bh Horizon Soil					
Leon	0.0015	0.0068	82	0.0021	31
Pomona	0.0040	0.0121	76	0.0023	19
LSD _{0.05}	0.0085	0.0099		0.0086	

Discussion

The importance of soluble organic P to P availability in soils revolves around whether it can be hydrolyzed to orthophosphate. In the main body of this dissertation it was shown that organic P makes up a significant portion of the total soluble P in Spodosols of the flatwoods. This appendix presents an initial investigation into the potential availability of the soluble organic P.

The results from this study demonstrated significant acid phosphatase activity in all soils. These data also demonstrated that greater phosphatase activity in the rhizosphere of slash pine seedlings than in the bulk soil. This suggests that production of acid phosphatases was stimulated in the rhizosphere of slash pine roots. Increased acid phosphatase activity has been observed in the rhizosphere of other plants (Boero and Thien 1979; Hedley et al. 1982; Tarafdar and Jungk 1987).

Several studies have shown that phosphatase activity is inversely related to the availability of orthophosphate (Speir and Ross 1978; Tabatabai 1982; Haynes and Swift 1988). The data from the rhizosphere, suggests that P fertilization inhibited phosphatase production by the slash pine. Phosphatase production in the roots of corn and sorghum was inhibited when adequate P was supplied to the plants (Furlani et al. 1984; Elliott and Lauchli 1986). Phosphorus fertilization also inhibited phosphatase activity

in soils supporting Douglas-fir stands in British Columbia (Pang and Kolenko 1978).

Although organic P can be utilized to a limited extent by certain plants, the vast majority of P is taken up in the inorganic form. The preceding acid phosphatase assay demonstrated that the potential to convert organic P to inorganic P was enhanced in the rhizosphere. However, unless the appropriate phosphatase is present to hydrolyze the specific organic P present in the soil, the organic P will remain relatively unavailable.

The constituent compounds comprising the soluble organic P fraction in most soils are not known (Anderson 1980). Inositol phosphates, nucleic acids and phospholipids generally comprise close to 50 % of the organic P in soils (Anderson 1980). The remaining half of the organic P is usually unidentified. In addition, the number of soils where organic P compounds have been investigated is small, giving little confidence in any generalization about the organic P make-up of soils.

In the second experiment, organic P accounted for 15 to 25 % of the water-soluble P released in two A horizon soils. In the two Bh horizon soils, organic P comprised over 75 % of the water-soluble P. However, no attempt was made to identify the forms of soluble organic P released in this study. Since the nature of the organic P in this study is unknown, only a limited discussion may be made concerning

the reference acid phosphatase added to water extracts. The significance of the data is that, in spite of the addition of adequate amounts of enzyme to hydrolyze all the organic P present, only 20 to 30 % of the organic P was hydrolyzed. This percentage was independent of the soil horizon from which the organic P was extracted. This indicates that there were several forms of organic P released in these soils and suggests that the mixture of organic P forms in the two horizons was similar. In a preliminary study, it was shown that the acid phosphatase used was able to hydrolyze phytic acid, guanosine-5'-monophosphate, and uridine-5-monophosphate. One can reasonably assume that monoesters such as these do not comprise the majority of the soluble organic P in these soils. The rest of the organic P is probably comprised of diester phosphates or resistant monoesters. The acid phosphatase added was, in fact not able to hydrolyze DNA indicating that phosphate diesters such as nucleic acids and phospholipids were probably not hydrolyzed. Although not hydrolyzed in this study, the diester portion of the soluble organic P pool may be relatively available to plants since diesterases are also common in soils (Speir and Ross 1978, Tabatabai 1982; Hayano 1987). Phosphorus contained in larger organic compounds such as humic and fulvic acids, would, however, probably not be readily available.

In summary, the results from the experiments presented in this paper suggest that a portion of the soluble organic P does contribute to the pool of plant-available P in Spodosols of the southeastern Coastal Plain. However, additional work is needed to identify the forms of organic P and types of phosphatases present in these spodosols. This work is needed to ascertain the role of organic P in plant nutrition and P cycling. This is particularly important in the Bh horizons, where the majority of the soluble P is in an organic form.

REFERENCES

- Adams, F., C. H. Burmester, N. V. Hue, and F. L. Long. 1980. A comparison of column displacement and centrifuge methods for obtaining soil solutions. *Soil Sci. Soc. Am. J.* 44:733-735.
- Alexander, T. G. and J. A. Robertson. 1972. EDTA extractable phosphorus in relation to available and inorganic phosphorus forms in soils. *Soil Sci.* 114:69-72.
- Allison, M. J. and H. M. Cook. 1981. Oxalate degradation by microbes of the large bowel of herbivores: The effect of dietary oxalate. *Science* 212:675-676.
- Anderson, G. 1980. Assessing organic phosphorus in soils. p. 411-431. *In* F. E. Khasawneh, E. C. Sample, and E. J. Kamprath (ed.) *The role of phosphorus in agriculture*. ASA, SSSA, and CSSA, Madison, WI.
- Anderson, G., E. G. Williams, and J. O. Moir. 1974. A comparison of the sorption of inorganic orthophosphate and inositol hexaphosphate by six acid soils. *J. Soil. Sci.* 25:51-62.
- Appelt, H., N. T. Coleman, and P. F. Pratt. 1975a. Interactions between organic compounds, minerals and ions in volcanic-ash-derived soils: I. Adsorption of benzoate, p-OH benzoate, salicylate and phthalate ions. *Soil Sci. Soc. Am. Proc.* 39:623-627.
- Appelt, H., N. T. Coleman, and P. F. Pratt. 1975b. Interactions between organic compounds, minerals and ions in volcanic-ash-derived soils: II. Effects of organic compounds on the adsorption of phosphate. *Soil Sci. Soc. Am. Proc.* 39:628-630.
- Arp, P. A. and W. L. Meyer. 1985. Formation constants for selected organo-metal (Al^{+3} , Fe^{+3})-phosphate complexes. *Can. J. Chem.* 63:3357-3366.
- Ballard, R. 1973. Extractability of reference phosphates by soil test reagents in absence and presence of soils. *Soil Crop Sci. Soc. Fla. Proc.* 33:169-174.

Ballard, R. and J. G. A. Fiskell. 1974. Phosphorus retention in Coastal Plain forest soils: I. Relationship to soil properties. *Soil Sci. Soc. Am. Proc.* 38:250-255.

Barber, S. A. 1984. Soil nutrient bioavailability. John Wiley & Sons, New York.

Barbour, M. G. and W. D. Billings (ed.) 1988. North American terrestrial vegetation. Cambridge Univ. Press, Cambridge.

Barnhisel, R. and P. M. Bertsch. 1982. Aluminum. *In* A. L. Page, R. H. Miller, and D. R. Keeney (ed.) *Methods of soil analysis*. Part 2. 2nd ed. *Agronomy* 9:275-300.

Barrow, N. J. 1987. Reactions with variable charge soils. Martinus Nijhoff Pub., Dordrecht, The Netherlands.

Bateman, D. F. and S. V. Beer. 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium rolfsii*. *Phytopath.* 55:204-211.

Berkheiser, V. E., J. J. Street, P. S. C. Rao, and T. L. Yuan. 1980. Partitioning of inorganic orthophosphate in soil-water systems. p. 179-224. *In* CRC Critical Reviews in Environmental Control. CRC Press, Boca Raton, FL.

Bloom, P. R. 1980. Phosphorus adsorption by an aluminum-peat complex. *Soil Sci. Soc. Am. J.* 45:267-272.

Bloomfield, C. 1953. A study of podzolization. Part I. The mobilization of iron and aluminum by Scots pine needles. *J. Soil Sci.* 4:5-17.

Boero, G. and S. Thien. 1979. Phosphatase activity and phosphorus availability in the rhizosphere of corn roots. p. 231-242. *In* J. L. Harley and R. S. Russel (ed.) *The soil-root interface*. Academic Press, New York.

Bowen, G.D. 1969. Nutrient status effects on loss of amides and amino acids from pine roots. *Plant Soil* 30:139-142.

Bruckert, S. 1970a. Influence des composés organiques solubles sur la pédogenèse en milieu acide. I. Etudes de terrain. *Ann. Agron.* 21:421-452.

Bruckert, S. 1970b. Influence des composés organiques solubles sur la pédogenèse en milieu acide. II. Expériences de laboratoire. Modalités d'action des agents complexants. *Ann. Agron.* 21:725-757.

Buol, S. B., F. D. Hole, and R. C. McCracken. 1980. Soil genesis and classification. Iowa State Univ. Press, Ames.

Burger, J. A. 1979. The effects of harvest and site preparation on the nutrient budget of an intensively managed southern pine forest ecosystem. Ph.D. diss. Univ. of Florida (Diss. Abstr. 40(9)-8005444).

Buurman, P. (ed.) 1984. Podzols. Van Nostrand Reinhold Co., New York.

Chen, Y. S. R., J. N. Butler, and W. Stumm. 1973a. Adsorption of phosphate on alumina and kaolinite from dilute aqueous solution. J. Colloid. Interface Sci. 43:421-436.

Chen, Y. S. R., J. N. Butler, and W. Stumm. 1973b. Kinetic study of phosphate reactions with aluminum oxide and kaolinite. Environ. Sci. Tech. 7:327-332.

Christensen, J. J. and R. M. Izatt. 1983. Handbook of metal ligand heats and related thermodynamic quantities. Marcel Dekker, Inc., New York.

Cole, C. V., G. S. Innis, and J. W. B. Stewart. 1977. Simulation of phosphorus cycling in semiarid grasslands. Ecology 58:1-15.

Coleman, N. T., J. T. Thorup, and W. A. Jackson. 1960. Phosphate sorption reactions that involve exchangeable Al. Soil Sci. 90:1-7.

Comerford, N. B. and M. F. Skinner. 1989. Residual phosphorus solubility for an acid, clayey, forested soil in the presence of oxalate and citrate. Can. J. Soil Sci. 69:111-117.

Comerford, N. B., G. Kidder, and A. V. Mollitor. 1984. Importance of subsoil fertility to forest and non-forest plant nutrition. p. 381-404. In E. L. Stone (ed.) Forest soils and treatment impacts. University of Tennessee, Knoxville.

Cosgrove, D. J. 1967. Metabolism of organic phosphates in soil. p. 216-228. In A. D. McLaren and G. H. Peterson (ed.) Soil biochemistry. Vol. 1. Marcel Dekker, Inc., New York.

Cotton, F. A., G. Wilkinson, and L. Gaus. 1987. Inorganic chemistry, 2nd. ed. John Wiley & Sons, New York.

Cromack, K. Jr, P. Sollins, W. C. Graustein, K. Speidel, A. W. Todd, G. Spycher, C. Y. Li, and R. L. Todd. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus Hysterangium crassum. Soil Biol. Biochem. 14:463-468.

Curl, E. A., and B. Truelove. 1986. The rhizosphere. Springer Verlag, Berlin.

Dean, L. A. and E. J. Rubins. 1947. Anion exchange in soils: I. Exchangeable phosphorus and the anion retention capacity. 63:377-387.

Deb, D. L. and N. P. Datta. 1967. Effects of associating anions on phosphorus retention in soil: I. Under variable phosphorus concentrations. Plant Soil 26:303-316.

Dighton, J. 1983. Phosphatase production by mycorrhizal fungi. Plant Soil 71:455-462.

Earl, K. D., J. K. Syers, and J. R. McLaughlin. 1979. Origin of the effects of citrate, tartrate, and acetate on phosphate sorption by soils and synthetic gels. Soil Sci. Soc. Am. J. 43:674-678.

Elgawhary, S. M., W. L. Lindsay, and W. D. Kemper. 1970. Effect of complexing agents and acids on the diffusion of zinc to a simulated root. Soil Sci. Soc. Am. Proc. 34:211-214.

Elkhatib, E. A., J. L. Hern, and T. E. Stanley. 1987. A rapid centrifugation method for obtaining soil solution. Soil Sci. Soc. Am. J. 51:578-583.

Elliott, G. C. and A. Lauchli. 1986. Evaluation of an acid phosphatase assay for detection of phosphorus deficiency in leaves of maize (Zea mays L.). J. Plant Nut. 9:1469-1477.

Fisher, R. F. 1972. Spodosol development and nutrient distribution under Hydnaceae fungal mats. Soil Sci. Soc. Am. Proc. 36:492-495.

Fisher, G. C. and O. L. Yam. 1984. Iron mobilization by heathland plant extracts. Geoderma 32:339-345.

Foy, C. D., E. H. Lee, and S. B. Wilding. 1987. Differential aluminum tolerances of two barley cultivars related to organic acids in their roots. J. Plant Nut. 10:1089-1101.

Furlani, A. M. C., R. B. Clark, J. W. Maranville, and W. M. Ross. 1984. Root phosphatase activity of sorghum genotypes grown with organic and inorganic sources of phosphorus. *J. Plant Nut.* 7:1583-1595.

Gardner, W. H. 1986. Water content. *In* A. Klute (ed.) *Methods of soil analysis. Part 1. Physical and mineralogical properties.* 2nd ed. *Agronomy* 9:493-544.

Gardner, W. K., D. G. Parbery, and D. A. Barber. 1982. The acquisition of phosphorus by *Lupinus albus* L. I. Some characteristics of the soil/root interface. *Plant Soil* 68:19-32.

Gardner, W. K., D. A. Barber, and D. G. Parbery. 1983. The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. *Plant Soil* 70:107-204.

Gholz, H. L., C. S. Perry, W. P. Cropper, Jr., and L. C. Hendry. 1985. Litterfall, decomposition, and nitrogen, and phosphorus dynamics in a chronosequence of slash pine (*Pinus elliottii*) plantations. *Forest Sci.* 31:463-478.

Goh, T. B. and P. M. Huang. 1984. Formation of hydroxy-Al-montmorillonite complexes as influenced by citric acid. *Can. J. Soil Sci.* 64:411-421.

Goh, T. B. and P. M. Huang. 1985. Changes in thermal stability and acidic characteristics of hydroxy-Al-montmorillonite complexes formed in the presence of citric acid. *Can. J. Soil Sci.* 65:519-522.

Goldberg, S. and G. Sposito. 1985. On the mechanism of specific phosphate adsorption by hydroxylated mineral surfaces: A review. *Comm. Soil Sci. Plant Anal.* 16:801-821.

Gomez, K. A. and A. A. Gomez. 1984. *Statistical procedures for agricultural research.* 2nd ed. John Wiley & Sons, New York.

Graustein, W. C., K. Cromack, Jr., and P. Sollins. 1977. Calcium oxalate: Occurrence in soils and effects on nutrient and geochemical cycles. *Science* 198:1252-1254.

Halstead, R. L. and R. B. McKercher. 1975. Biochemistry and cycling of phosphorus. p. 31-63. *In* E. A. Paul and A. D. McLaren (ed.) *Soil biochemistry.* Vol. 4. Marcel Dekker, Inc., New York.

Harder, W. 1973. Microbial metabolism of organic C1 and C2 compounds. *Antonie van Leeuwenhoek* 39:650-652.

Hayano, K. 1987. Characterization of a phosphodiesterase component in a forest soil extract. *Biol. Fertil. Soils* 3:159-164.

Hayman, D. S. 1975. Phosphorus cycling by soil micro-organisms and plant roots. p. 67-91. In N. Walker (ed.) *Soil microbiology: A critical review*. Rothamsted Exp. Sta., Harpenden, Hertfordshire, England.

Haynes, R. J. and R. S. Swift. 1985. Effects of air-drying on the adsorption and desorption of phosphate and levels of extractable phosphate in a group of acid soils, New Zealand. *Geoderma* 35:145-157.

Haynes, R. J. and R. S. Swift. 1988. Effects of lime and phosphate additions on changes in enzyme activities, microbial biomass and levels of extractable nitrogen, sulfur and phosphorus in an acid soil. *Biol. Fertil. Soils* 6:153-158.

Hedley, M. J., R. E. White, and P. H. Nye. 1982. Plant induced changes in the rhizosphere of rape (Brassica napus var emerald) seedlings. III. Changes in the L value, soil phosphate fraction and phosphatase activity. *New Phytol.* 91:45-56.

Hingston, F. J., R. J. Atkinson, A. M. Posner, and J. P. Quirk. 1967. Specific adsorption of anions. *Nature* 215:1459-1461.

Hingston, F. J., A. M. Posner, and J. P. Quirk. 1970. Anion binding at oxide surfaces - The adsorption envelope. *Search* 1:324-327.

Hingston, F. J., A. M. Posner, and J. P. Quirk. 1972. Anion adsorption by goethite and gibbsite. I. The role of the proton in determining adsorption envelopes. *J. Soil Sci.* 23:177-192.

Hingston, F. J., A. M. Posner, and J. P. Quirk. 1974. Anion adsorption by goethite and gibbsite. II. Desorption of anions from hydrous oxide surfaces. *J. Soil Sci.* 25:16-26.

Hodgkinson, A. 1977. *Oxalic acid in biology and medicine*. Academic Press, London.

Hsu, P. H. 1965. Fixation of phosphate by aluminum and iron in acid soils. *Soil Sci.* 99:398-403.

Huang, P. M. and M. Schnitzer (ed.) 1986. Interactions of soil minerals with natural organics and microbes. *SSSA Spec. Publ.* 17. SSSA, Madison, WI.

- Huang, P. M. and A. Violante. 1986. Influence of organic acids on crystallization and surface properties of precipitation products of aluminum. p. 159-221. In P. M. Huang and M. Schnitzer (ed.) Interactions of soil minerals with natural organics and microbes. SSSA Spec. Publ. 17. SSSA, Madison, WI.
- Hue, N. V., G. R. Craddock, and F. Adams. 1986. Effect of organic acids on aluminum toxicity in subsoils. *Soil Sci. Soc. Am. J.* 50:28-34.
- Humphreys, F. R. and W. L. Pritchett. 1971. Phosphorus adsorption and movement in some sandy forest soils. *Soil Sci. Soc. Am. Proc.* 35:495-500.
- Inoue, K. and P. M. Huang. 1984. Influence of citric acid on the natural formation of imogolite. *Nature* 308:58-60.
- Inskeep, W. P. and J. Baham. 1983. Competitive complexation of Cd(III) and Cu(II) by water soluble organic ligands and Na-montmorillonite. *Soil Sci. Soc. Am. J.* 47:1109-1115.
- Inskeep, W. P. and S. D. Comfort. 1986. Thermodynamic predictions for the effects of root exudates on metal speciation in the rhizosphere. *J. Plant Nut.* 9:567-586.
- Jalal, M. A. F. and D. J. Read. 1983. The organic acid composition of Calluna heathland soil with special reference to phyto- and fungitoxicity. I. Isolation and identification of organic acids. *Plant Soil* 70:257-272.
- James, B. R. and S. J. Riha. 1989. Aluminum leaching by mineral acids in forest soils: II. Role of the forest floor. *Soil Sci. Soc. Am. J.* 53:264-269.
- Jauregui, M. A. and H. M. Reisenauer. 1982. Dissolution of oxides of manganese and iron by root exudate components. *Soil Sci. Soc. Am. J.* 46:314-317.
- Johnson, D. W., J. Turner, and J. M. Kelly. 1982. The effects of acid rain on forest nutrient status. *Water Resour. Res.* 18:449-461.
- Jungk, A. 1987. Soil-root interactions in the rhizosphere affecting plant available phosphorus. *J. Plant Nut.* 10:1197-1204.
- Jurinak, J. J., L. M. Dudley, M. F. Allen, and W. G. Knight. 1986. The role of calcium oxalate in the availability of phosphorus in soils of semiarid regions: A thermodynamic study. *Soil Sci.* 142:255-261.

Kafkafi, U., A. M. Posner, and J. P. Quirk. 1967. Desorption of phosphate from kaolinite. *Soil Sci. Soc. Am. Proc.* 31:348-353.

Kaurichev, I. S., T. N. Ivanova, and Ye. M. Nozdrunova. 1963. Low-molecular organic acid content of water-soluble organic matter in soils. *Soviet Soil Sci.* 223-229.

Klute, A. (ed.) 1986. *Methods of soil analysis. Part 1.* 2nd ed. Agronomy 9. ASA & SSSA, Madison, WI.

Kowalenko, C. G. 1978. Organic nitrogen, phosphorus and sulfur in soils. p. 95-136. *In* M. Schnitzer and S. U. Khan (ed.) *Soil organic matter.* Elsevier Scientific Publishing Co., Amsterdam.

Krafczyk, I., G. Trolldenier, and H. Beringer. 1984. Soluble root exudates of maize: Influence of potassium supply and rhizosphere microorganisms. *Soil Biol. Biochem.* 16:315-322.

Kuiters, A. T. and H. M. Sarink. 1986. Leaching of phenolic compounds from leaf and needle litter of several deciduous and coniferous trees. *Soil Biol. Biochem.* 18:475-480.

Kuo, S. and E. G. Lotse. 1974. Kinetics of phosphate adsorption and desorption by hematite and gibbsite. *Soil Sci.* 116:400-406.

Kurtz, T., E. E. DeTurk, and R. H. Bray. 1946. Phosphate adsorption by Illinois soils. *Soil Sci.* 61:11-124.

Lajtha, K. and W. H. Schlesinger. 1988. The biochemistry of phosphorus cycling and phosphorus availability along a desert soil chronosequence. *Ecology* 69:24-39.

Lee, D. P. and A. D. Lord. 1986. A high performance phase for the organic acids. *LC-GC.* 5:261-265.

Levesque, M. and M. Schnitzer. 1967. Organo-metallic interactions in soils: 6. Preparation and properties of fulvic acid-metal phosphates. *Soil Sci.* 103:183-190.

Lindsay, W. L. 1979. *Chemical equilibria in soils.* John Wiley & Sons, New York.

Lopez-Hernandez, D., F. G. Siegert, and J. V. Rodriguez. 1979. The effect of some organic anions on phosphate removal from acid and calcareous soils. *Soil Sci.* 128:321-326.

Low, P. F. and C. A. Black. 1950. Reactions of phosphate with kaolinite. *Soil Sci.* 70:273-290.

Lynch, J. M. 1983. Soil biotechnology. Microbiological factors in crop productivity. Blackwell Scientific Pub., Oxford.

Malajczuk, N., and K. Cromack, Jr. 1982. Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of Pinus radiata and Eucalyptus marginata. New Phytol. 92:527-531.

Marschner, H., V. Romheld, W. J. Horst, and P. Martin. 1986. Root induced changes in the rhizosphere: Importance for the mineral nutrition of plants. Z. Pflanz. Bodenk. 149:441-456.

Martell, A. E. and R. M. Smith. 1977. Critical stability constants. Vol. 3: Other organic ligands. Plenum Press, New York.

Martell, A. E. and R. M. Smith. 1982. Critical stability constants. Vol. 5: First supplement. Plenum Press. New York,

Martell, A. E., R. J. Motekaitis, and R. M. Smith. 1988. Structure-stability relationships of metal complexes and metal speciation in environmental aqueous solutions. Environ. Tox. Chem. 7:417-434.

Martin, A. E. and R. Reeve. 1960. Chemical studies of podzolic illuvial horizons. IV. The flocculation of humus by aluminum. J. Soil Sci. 11:369-381.

Martin, J. K. 1964. Soil organic phosphorus. III: Patterns of organic phosphorus in a series of New Zealand soil. N. Z. J. Agric. Res. 7:750-760.

Martin, J. K. 1970. Organic phosphate compounds in water extracts of soils. Soil Sci. 109:362-375.

Martin, J. P. and K. Haider. 1986. Influence of mineral colloids on turnover rates of soil organic carbon. p. 283-304. In P. M. Huang and M. Schnitzer (ed.) Interactions of soil minerals with natural organics and microbes. SSSA Spec. Publ. 17. SSSA, Madison, WI.

Maxwell, D. P. and D. F. Bateman. 1968, Oxalic acid biosynthesis by *Sclerotium rolfsii*. Phytopath. 58:1635-1642.

McColl, J. G. and A. A. Pohlman. 1986. Soluble organic acids and their chelating influence on Al and other metal dissolution from forest soils. Water Air Soil Pol. 31:917-927.

McColl, J. G., A. A. Pohlman, J. M. Jersak, S. C. Tam, and R. R. Northup. Organics and metal solubility in forest soils. In S. P. Gessel (ed.) Proceedings of the 7th North American Forest Soils Conf. Univ. of British Columbia Press, Vancouver. (In press).

McKeague, J. A., M. V. Cheshire, F. Andreux, and J. Berthelin. 1986. Organo-mineral complexes in relation to pedogenesis. p. 549-592. In P. M. Huang and M. Schnitzer (ed.) Interactions of soil minerals with natural organics and microbes. SSSA Spec. Publ. 17. SSSA, Madison, WI.

Miller, W. P., L. W. Zelazny, and D. C. Martens. 1986. Dissolution of synthetic crystalline and noncrystalline iron oxides by organic acids. *Geoderma* 37:1-13.

Morris, L. A. 1981. Redistribution and mobilization of nutrients as a result of harvest and site preparation of a pine flatwoods forest. Ph.D. diss. Univ. of Florida. (Diss. Abstr. 43(1) 82113683).

Motekaitis, R. J. and A. E. Martell. 1984. Complexes of aluminum (III) with hydroxy carboxylic acids. *Inorg. Chem.* 23:18-23.

Muir, J. W., R. I. Morrison, C. J. Bown, and J. Logan. 1964a. The mobilization of iron by aqueous extracts of plants. I. Composition of the amino acid and organic acid fractions of an aqueous extract of pine needles. *J. Soil Sci.* 15:220-225.

Muir, J. W., J. Logan, and C. J. Bown. 1964b. The mobilization of iron by aqueous extracts of plants. II. Capacities of the amino-acid and organic-acid fractions of a pine-needle extract to maintain iron in solution. *J. Soil Sci.* 15:226-237.

Murphy, J. and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27:31-36.

Nagarajah, S., A. M. Posner, and J. P. Quirk. 1968. Desorption of phosphate from kaolinite by citrate and bicarbonate. *Soil Sci. Soc. Am. Proc.* 32:507-510.

Nagarajah, S., A. M. Posner, and J. P. Quirk. 1970. Competitive adsorption of phosphate with polygalacturonate and other organic anions on kaolinite and oxide surfaces. *Nature* 228:83-85.

Nanzyo, M. 1984. Diffuse reflectance infrared spectra of phosphate sorbed on alumina gel. *J. Soil Sci.* 35:63-69.

Nanzyo, M. and Y. Watanabe. 1984. Mechanisms of phosphate sorption on soil components with high phosphate retention capacity. *JARQ* 18:87-91.

Neary, D. G., E. J. Jokela, N. B. Comerford, S. R. Colbert, and T. E. Cooksey. Understanding competition for soil nutrients - The key to site productivity on southeastern Coastal Plain Spodosols. In S. P. Gessel (ed.) *Proceedings of the 7th North American Forest Soils Conf.* Univ. of British Columbia Press, Vancouver. (In press).

Ng Kee Kwong, K. F. and P. M. Huang. 1977. Influence of citric acid on the hydrolytic reactions of aluminum. *Soil Sci. Soc. Am. J.* 41:692-697.

Nye, P. H. 1984. On estimating the uptake of nutrients solubilized near roots or other surfaces. *J. Soil Sci.* 35:439-446.

Nye, P. H. and P. B. Tinker. 1977. *Solute movement in the soil-root system.* Univ. California Press, Berkeley.

O'Keefe, D. M., T. R. Fox, and M. E. Will. 1987. An evaluation of microbial inhibitors in P sorption studies. p. 189. In *Agronomy abstracts.* ASA, Madison, WI.

Page, A. L., R. H. Miller, and D. R. Keeney (ed.) 1982. *Methods of soil analysis. Part 2.* 2nd ed. Agronomy 9. ASA & SSSA, Madison, WI.

Pang, P. C. K. and H. Kolenko. 1986. Phosphomonoesterase activity in forest soils. *Soil Biol. Biochem.* 18:35-40.

Parfitt, R. L., A. R. Fraser, J. D. Russell, and V. C. Farmer. 1977. Adsorption on hydrous oxides. II. Oxalate, benzoate and phosphate on gibbsite. *J. Soil Sci.* 28:40-47.

Perrin, D. D. 1979. *Stability constants of metal-ion complexes: Part B, Organic ligands.* Pergamon Press, Oxford.

Phillips, L. P., N. B. Comerford, D. G. Neary, and R. S. Mansell. Distribution and simulation of soil water above a shallow fluctuating water table in a forested Spodosol. *Soil Sci. Soc. Am. J.* In press.

Pierre, W. H. and F. W. Parker. 1927. *Soil phosphorus studies: II. The concentration of organic and inorganic phosphorus in the soil solution and soil extracts and the availability of the organic phosphorus to plants.* *Soil Sci.* 24:119-128.

- Pohlman, A. A. and J. G. McColl. 1986. Kinetics of metal dissolution from forest soils by soluble organic acids. *J. Environ. Qual.* 15:86-92.
- Pohlman, A. A. and J. G. McColl. 1988. Soluble organics from forest litter and their role in metal dissolution. *Soil Sci. Soc. Am. J.* 52:265-271.
- Ponomereva, V. V. 1969. Theory of podzolization. Israel Program for Scientific Translations, Jerusalem.
- Pritchett, W. L. and N. B. Comerford. 1983. Nutrition and fertilization of slash pine. p. 69-90. *In* E. L. Stone (ed.) The managed slash pine ecosystem. Symposium Proc., Gainesville, FL. 9 June - 11 June 1981. Univ. Florida, Gainesville.
- Reddy, K. R., T. C. Feijtel, and W. H. Patrick, Jr. 1986. Effect of soil redox conditions on microbial oxidation of organic matter. p. 117-156. *In* Y. Chen and Y. Avnimelech (ed.) The role of organic matter in modern agriculture. Martinus Nijhoff Pub. Dordrecht, The Netherlands.
- Reid, R. K., C. P. P. Reid, and P. J. Szaniszlo. 1985. Effects of synthetic and microbially produced chelates on the diffusion of iron and phosphorus to a simulated root in soil. *Biol. Fertil. Soils* 1:45-52.
- Rovira, A. D. and C. B. Davey. 1974. Biology of the rhizosphere. p. 153-204. *In* E. W. Carson (ed.) The plant root and its environment. University Press of Virginia, Charlottesville, VA.
- Rovira, A. D. and B. M. McDougall. 1967. Microbial and biochemical aspects of the rhizosphere. p. 417-463. *In* A. D. McLaren and G. H. Peterson (ed.) Soil biochemistry, Volume 1. Marcel Dekker, Inc., New York.
- Rozycki, H. and E. Strzelczyk. 1986. Organic acids production by Streptomyces spp. isolated from soil, rhizosphere and mycorrhizosphere of pine (Pinus sylvestris L.). *Plant Soil* 96:337-345.
- Ryden, J. C. and J. K. Syers. 1975. Rationalization of ionic strength and cation effects on phosphate sorption by soils. *J. Soil Sci.* 26:395-406.
- Ryden, J. C. and J. K. Syers. 1977. Origin of the labile phosphate pool in soils. *Soil Sci.* 123:353-361.

Schnitzer, M. and S. I. M. Skinner. 1985. Organo-metallic interactions in soils: 4. Carboxyl and hydroxyl groups in organic matter and metal retention. *Soil Sci.* 99:278-284.

Schwartz, S. M., J. E. Varner, and W. P. Martin. 1954. Separation of organic acids from several dormant and incubated Ohio soils. *Soil Sci. Soc. Am. Proc.* 18:174-177.

Schwertmann, U., H. Kodama, and W. R. Fisher. 1986. Mutual interactions between organics and iron oxides. p. 223-250. In P. M. Huang and M. Schnitzer (ed.) *Interactions of soil minerals with natural organics and microbes*. SSSA Spec. Publ. 17. SSSA, Madison, WI.

Sillen, L. G. and A. E. Martell. 1971. Stability constants of metal-ion complexes. Supplement No. 1. Special Pub. 25. The Chemical Society, London.

Sinha, M. K. 1971a. Organo-metallic phosphates. I. Interaction of phosphorus compounds with humic substances. *Plant Soil* 35:471-484.

Sinha, M. K. 1971b. Organo-metallic phosphates. II. Physico-chemical characteristics of humic and fulvic metal phosphates. *Plant Soil* 35:484-493.

Smeck, N. E. 1985. Phosphorus dynamics in soils and landscapes. *Geoderma* 36:185-199.

Smith, W. H. 1969. Release of organic materials from the roots of tree seedlings. *Forest Sci.* 15:138-143.

Smith, W. H. 1976. Character and significance of forest tree root exudates. *Ecology* 57:324-331.

Sollins, P., K. Cromack, Jr., R. Fogel, and C. Y. Li. 1981. Role of low-molecular-weight organic acids in the inorganic nutrition of fungi and higher plants. p. 607-619. In D. T. Wicklow and G. C. Carroll (ed.) *The fungal community*. Marcel Dekker, Inc., New York.

Song, S. K. and P. M. Huang. 1988. Dynamics of potassium release from potassium-bearing minerals as influenced by oxalic and citric acids. *Soil Sci. Soc. Am. J.* 52:383-390.

Sparks, D. L. 1986. Kinetics of reactions in pure and mixed systems. p. 83-146. In D. L. Sparks (ed.) *Soil physical chemistry*. CRC Press, Boca Raton, FL.

Speir, T. W. and D. J. Ross. 1978. Soil phosphatases and sulphatases. p. 197-249. In E. G. Burns (ed.) *Soil enzymes*. Academic Press, New York.

Sposito, G. 1984. The surface chemistry of soils. Oxford Univ. Press, New York.

Sposito, G. and S. V. Mattigod. 1980. GEOCHEM: A computer program for the calculation of chemical equilibria in soil solutions and other natural water systems. The Kearney Foundation of Soil Science, Riverside, CA.

Sposito, G., F. T. Bingham, S. S. Yadav, and C. A. Inouye. 1980. Trace metal complexation by fulvic acid extracted from sewage sludge: II. Development of chemical models. Soil Sci. Soc. Am. J. 46:51-56.

Stainton, M. P. 1980. Errors in molybdenum blue methods for determining orthophosphate in freshwater. Can. J. Fish. Aquat. Sci. 37:472-478.

Stevenson, F. J. 1967. Organic acids in soil. p. 119-146. In A. D. McLaren and G. H. Peterson (ed.) Soil biochemistry. Vol 1. Marcel Dekker, Inc., New York.

Stevenson, F. J. 1982. Humus chemistry. Genesis, composition, reactions. John Wiley & Sons, New York.

Stevenson, F. J. 1986. Cycles of soil: Carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley & Sons, New York.

Struthers, P. H. and D. H. Sieling. 1950. Effects of organic anions on phosphate precipitation by iron and aluminum as influenced by pH. Soil Sci. 69:205-213.

Stumm, W. 1986. Coordination interactions between soil solids and water - An aquatic chemist's point of view. Geoderma 38:19-30.

Stumm, W. and J. J. Morgan. 1981. Aquatic chemistry. 2nd ed. John Wiley & Sons, New York.

Swenson, R. M., C. V. Cole, and D. H. Sieling. 1949. Fixation of phosphate by iron and aluminum and replacement by organic and inorganic ions. Soil Sci. 67:3-22.

Swindel, B. F. 1970. Some applications of the principle of conditional error. USDA Forest Service Res. Pap. SE-59. Asheville, NC.

Syers, J. K. and I. K. Iskandar. 1981. Soil phosphorus chemistry. p. 571-599. In I. K. Iskandar (ed.) Modeling wastewater renovation: Land treatment. John Wiley & Sons, New York.

Tabatabai, M. A. 1982. Soil enzymes. In A. L. Page, R. H. Miller, and D. R. Keeney (ed.) Methods of soil analysis. Part 2. 2nd ed. Agronomy 9:903-948.

Tam, S. C. 1987. Simulated acid rain and the importance of organic ligands on the availability of aluminum in soil. Water Air Soil Pol. 36:193-206.

Tan, K. H. 1986. Degradation of soil minerals by organic acids. p. 1-28. In P. M. Huang and M. Schnitzer (ed.) Interactions of soil minerals with natural organics and microbes. SSSA Spec. Publ. 17. SSSA, Madison, WI.

Tarafdar, J. C. and N. C. Claassen. 1988. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol. Fertil. Soils 5:308-312.

Tarafdar, J. C. and A. Jungk. 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol. Fertil. Soils 3:199-204.

Tarapchak, S. J., S. M. Bigelow, and C. Rubitschun. 1982. Overestimation of orthophosphorus concentrations in surface waters of southern Lake Michigan: Effects of acid and ammonium molybdate. Can. J. Fish. Aquat. Sci. 39:296-304.

Tate, K. R. 1984. The biological transformation of P in soil. Plant Soil 76:245-256.

Taylor, A. W. and E. L. Gurney. 1962. Solubility of amorphous aluminum phosphate. Soil Sci. 67:3-22.

Traina, S. J., G. Sposito, D. Hesterberg, and U. Kafkafi. 1986a. Effects of pH and organic acids on orthophosphate solubility in an acidic, montmorillonitic soil. Soil Sci. Soc. Am. J. 50:45-52.

Traina, S. J., G. Sposito, D. Hesterberg, and U. Kafkafi. 1986b. Effects of ionic strength, calcium, and citrate on orthophosphate solubility in an acidic, montmorillonitic soil. Soil Sci. Soc. Am. J. 50:623-627.

Traina, S. J., G. Sposito, G. R. Bradford, and U. Kafkafi. 1987. Kinetic study of citrate effects on orthophosphate solubility in an acidic, montmorillonitic soil. Soil Sci. Soc. Am. J. 51:1483-1487.

Turner, J. and M. J. Lambert. 1985. Soil phosphorus forms and related tree growth in a long term Pinus radiata phosphate fertilizer trial. Comm. Soil Sci. Plant Anal. 16:275-288.

Vancura, V. 1964. Root exudates of plants. I. Analysis of root exudates of barley and wheat in their initial phases of growth. *Plant Soil* 21:231-248.

Vancura, V. and A. Hovadik. 1965. Root exudates of plants. II. Composition of root exudates of some vegetables. *Plant Soil* 22:21-32.

Van Rees, K. C. J. and N. B. Comerford. 1986. Vertical root distribution and strontium uptake of a slash pine stand on a flatwoods Spodosol. *Soil Sci. Soc. Am. J.* 50:1042-1046.

Van Riemsdijk, W. H., L. J. M. Bourans, and F. A. M. De Haan. 1984. Phosphate sorption by soils: I. A model for phosphate reaction with metal-oxides in soil. *Soil Sci. Soc. Am. J.* 48:537-541.

Veith, J. A. and G. Sposito. 1977. Reactions of aluminosilicates, aluminum hydrous oxides, and aluminum oxides with o-phosphate: The formation of x-ray amorphous analogs of variscite and montebrazite. *Soil Sci. Soc. Am. J.* 41:870-876.

Whitehead, D. C. 1964. Identification of p-hydroxybenzoic, vanillic, p-coumaric and ferulic acids in soils. *Nature* 202:417-418.

Wild, A. and O. L. Oke. 1966. Organic phosphate compounds in calcium chloride extracts of soils: Identification and availability to plants. *J. Soil Sci.* 17:356-371.

Wood, T., F. H. Bormann, and G. K. Voigt. 1984. Phosphorus cycling in a northern hardwood forest: Biology and chemical control. *Science* 223:391-393.

Yuan, T. L. 1966. Characteristics of surface and subsurface spodic horizons of some Spodosols. *Soil Crop Sci. Soc. Fla. Proc.* 26:163-174.

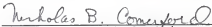
BIOGRAPHICAL SKETCH

I've studied now Philosophy
And Jurisprudence, Medicine,
And even, alas! Theology
All through and through with ardour keen!
Here now I stand, poor fool, and see
I'm just as wise as formerly.
Am called a Master, even Doctor too,
And now I've nearly ten years through
Pulled my students by their noses to and fro
and up and down, across, about
And see there's nothing we can know!
Faust

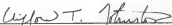
Thomas Robert Fox was born on May 7, 1958 in Lakewood, Ohio to John R. and Ruth F. Fox. He grew up in northern Ohio and remains a diehard fan of the Cleveland Indians through 30+ years of losing seasons. He enrolled at the University of Maine and received a B. S. in forestry in 1980 and a Certificate of Advanced Study in pulp and paper technology in 1981. He remains a loyal Black Bear fan through 10+ years of losing seasons. While in Maine, he was employed as a project forester by International Paper Company. In 1984, he completed a M.S. in forest soils at Virginia Polytechnic Institute and State University. Realizing enough is enough, he never became a Hokie fan. After receiving his M.S. degree, he worked for the Forest Nutrition Cooperative at North Carolina State University and then for Weyerhaeuser

Company in Centralia, Washington. In January 1986, he was admitted to the Graduate School of the University of Florida to work toward his Ph.D. degree in forest soils in the Soil Science Department. He is currently employed as the silvicultural research coordinator for ITT Rayonier, Inc., Southeast Forest Resources in Fernandina Beach, Florida.


I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Nicholas B. Comerford, Chairman
Associate Professor of Soil Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Clifford T. Johnston
Assistant Professor of Soil Science


I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Brian L. McNeal
Professor of Soil Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


C. P. Patrick Reid
Professor of Forest Resources and
Conservation

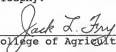
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Earl L. Stone
Professor of Soil Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1989



Dean, College of Agriculture

Dean, Graduate School

